Selectivity of *Escherichia coli* RNA Polymerase for Template Conformation

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ABSTRACT: *Escherichia coli* RNA polymerase (RNAP) exhibits a strong selectivity for the secondary structure of its template DNA, as shown by the influence both of the DNA conformation on the transcription cycle and of the enzyme on the DNA conformation itself. Binding, chain initiation and elongation characteristics of RNAP, and DNA conformational characteristics were examined by use of the alternating copolymer poly(dGdm^{5}C)·poly(dGdm^{5}C) as template. Transcription is impeded when the DNA is in the Z conformation as compared with the B; the initial conformation is determined by the concentration of the conformational effectors Mg^{2+} and $[Co(NH_3)_6]^{3+}$. RNAP binds to both Z and B conformers; the total binding is moderately greater when the template is in the B conformation than when it is strongly stabilized in the Z, by $[Co(NH_3)_6]^{3+}$ concentrations much higher than those required for B–Z transition. However, the Z conformer is much more easily displaced competitively from the bulk of its complexes with RNAP than is the B, indicating a specific binding preference for the B conformer. When the template is in the B conformation, or is moderately stabilized in the Z by Mg^{2+} concentrations such that the polynucleotide is just fully converted from B to Z, elongation is predicted well by chain initiation, indicating that on the Z conformer RNAP is effectively inhibited at the chain initiation or at an earlier stage. The average chain growth rates for polymeric product synthesized on B and on moderately stabilized Z are similar, even though overall RNA synthesis is considerably lowered on the Z form, again indicating that the limiting events precede elongation. When the Z conformer is strongly stabilized, chain initiation and elongation are further inhibited. Elongation is still roughly correlated with chain initiation, but some additional inhibition of elongation takes place independently. Circular dichroism analysis shows that RNAP–DNA binding affects the B–Z conformational equilibrium, leading to reformation of the B conformer from Z and interference with conversion of B to Z, under conditions that would otherwise favor the Z conformer. Thus, there is an RNAP concentration dependent shift of the B–Z transition to higher concentrations of Z-inducing cation, and there is an RNAP concentration dependent decrease in the rate of B to Z conversion. These effects were observed for poly(dGdm^{5}C)·poly(dGdm^{5}C), with Z stabilized by $[Co(NH_3)_6]^{3+}$ or Mg^{2+}. (They were observed as well for the unmethylated copolymer poly(dGdC)·poly(dGdC), with Z stabilized by $[Co(NH_3)_6]^{3+}$.) Perturbation of the Z conformer was detectable by circular dichroism at an RNAP:polynucleotide ratio down to a practical limit of ~1 RNAP:500 bp. Interference with B to Z conversion was readily detected at 1 RNAP:500 bp. Both of these phenomena appear to be fairly specific for the holoenzyme ($\alpha_2\beta\beta'\sigma$) form of RNAP, with the core enzyme ($\alpha_2\beta\beta'$) giving much smaller effects.

The specific interaction of regions of DNA with particular proteins involves recognition of particular sequences in the DNA. Since certain sequences are readily converted from the common B conformation to other conformations, including the left-handed Z, it is likely that the proteins recognize conformations as well. Besides the changes in specific protein interaction with DNA regions due to supercoiling (reviewed by Giaver et al. (1988)), substantial effects due to the DNA secondary structure itself are also known. For example, an A-type conformation rather than a B has been found to be adopted by the region specifically binding eukaryotic TF-IIIA (McCall et al., 1986; Rhodes & Klug, 1986), and the recognition region for *BclI* nuclease has a bent conformation (Banks et al., 1989).

We had shown previously (Butzow et al., 1984) that poly(dGdC)·poly(dGdC) and poly(dGdm^{5}C)·poly(dGdm^{5}C) are substantially poorer templates for *Escherichia coli* RNA polymerase (RNAP)[1] when they are in the Z conformation, in comparison with the B, with the activity of the enzyme on the template declining in correspondence with the degree of conversion to the Z form, induced by Mg^{2+} or $[Co(NH_3)_6]^{3+}$. Jovin and colleagues (Durand et al., 1983; van de Sande & Jovin, 1982) showed that wheat germ RNA polymerase II transcribed poorly the Z forms of these polymers induced by Mg^{2+} or solvent effects. These studies did not determine at what point or points in the transcription process the "wrong" template conformation is inhibitory: is the binding of polymerase to template involved, or initiation of RNA synthesis, or elongation? Two subsequent studies of elongation have implicated this stage: Peck and Wang's (1985) study of transcription by RNAP through a tract of (dCdG)·(dGdC) inserted into a coding sequence and that of Job et al. (1988) on primer extension by wheat germ RNA polymerase II using dGdC copolymers as templates. In this paper we address the question of which stages in transcription are affected, through measurement and comparison of template binding, chain initiation, and elongation by RNAP; and we also determine whether RNAP binding to template can change a conformation unfavorable for transcription into a more favorable one, as preliminary evidence (Butzow et al., 1984) has suggested.

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[1] Abbreviations: CD, circular dichroism; DTT, dithiothreitol; EDTA, ethylenediaminetetracetate; RNAP, RNA polymerase (*E. coli*); Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol.

The results show that binding and chain initiation, as well as elongation, are decreased on the Z conformer, with chain initiation and even binding limiting later events, and that RNAP binding itself leads to destabilization of the Z conformer in favor of the B.

We use a template that is interconvertible between the B and Z forms, the alternating copolymer poly(dGdm^{5}C)·poly(dGdm^{5}C). This polynucleotide has a more stable Z form (Behe & Felsenfeld, 1981; Fujii et al., 1982) than even poly(dGdC)·poly(dGdC) (Wang et al., 1979). Mg^{2+} and $[Co(NH_3)_6]^{3+}$ at relatively low concentrations are employed as the agents to stabilize the Z conformation.

Experimental Procedures

RNA Polymerase. E. coli RNA polymerase was prepared according to Burgess (Burgess & Jendrisak, 1975) with the modification of Lowe et al. (1979) and then further purified according to Gonzales et al. (1977) to eliminate core enzyme ($\alpha_2\beta\beta'$) not complexed with σ. Core enzyme was prepared according to Lowe et al. (1979) by Dr. P. Chuknyisky in our laboratory. Both the holoenzyme ($\alpha_2\beta\beta'\sigma$) and the parent, non-$\sigma$-saturated, preparations typically exhibited about 35% active core when assayed on T7 DNA according to the method of Chamberlin (Chamberlin et al., 1979, 1983), and the enzyme preparations were routinely diluted to 200 μM before addition to the assay reaction. We have subsequently found that if RNAP is introduced directly into the assay reaction, the measured activity is increased 2-fold or more (J.J.B., R. G. Stankis, and G.L.E., unpublished results), indicating that the preparations used for the present study contain 70–80% active enzyme molecules.

Polynucleotides. Poly(dGdm^{5}C)·poly(dGdm^{5}C), poly(dGdC)·poly(dGdC), and poly(dAdT)·poly(dAdT) were obtained from Pharmacia-PL. They were dissolved and equilibrated in 50 mM NaCl/5 mM Tris buffer, pH 8, by gentle rotation at 5 °C for several days. Poly(dGdm^{5}C)·poly(dGdm^{5}C) and poly(dGdC)·poly(dGdC) were freed from bound di- and polyvalent metals by passage over Chelex-100 (Na form, Bio-Rad). For particular experiments, poly(dGdm^{5}C)·poly(dGdm^{5}C) was then purified by gel electrophoresis (as described below) to eliminate chains shorter than 1300 bp. For filter retention experiments, the sized poly(dGdm^{5}C)·poly(dGdm^{5}C) was 5′-end-labeled by transfer of the ^{32}P-labeled phosphate from [γ-^{32}P]ATP (Maniatis et al., 1982; conditions for blunt-ended DNA), to ~3000 Ci/mol ends. Unless the shorter chains are removed from the poly(dGdm^{5}C)·poly(dGdm^{5}C) preparation, the chain-length distribution of RNA product synthesized by *E. coli* RNA polymerase contains, already at early times of reaction, a considerable amount of label migrating at long chain lengths. We found that elimination of poly(dGdm^{5}C)·poly(dGdm^{5}C) chains shorter than ~1300 bp effectively eliminates the anomalous long-chain product, which almost certainly represents the result of covalent addition of ribonucleotides to template (see Nath and Hurwitz (1974)). Poly(dGdm^{5}C)·poly(dGdm^{5}C) of 800–1300 bp still supports synthesis of the anomalous product.

Gel Electrophoretic Fractionation of Poly(dGdm^{5}C)·Poly(dGdm^{5}C). In order to resolve moderate quantities of poly(dGdm^{5}C)·poly(dGdm^{5}C) into narrower subdistributions, we dispersed up to 1 mmol (res) of the unresolved polynucleotide by electrophoresis on 1.5% agarose gels, in TBE buffer (100 mM Tris, 95 mM boric acid, 2 mM EDTA). Gels were cut at the ~800- and ~1300-bp positions on the basis of migration of restriction markers. Polynucleotides were extracted from the resulting gel strips by electrophoresis into a low ionic strength buffer (5 mM Tris, 2.5 mM acetic acid) in dialysis bags for several hours on a horizontal electrophoresis tray, which was followed by reversal of the current polarity for ~10 min. Extracted polynucleotides were freed from the soluble gel matrix on NACS columns (NACS "prepac" from BRL Laboratories, Bethesda, MD) after pressure concentration of the polynucleotide solution on a membrane device (Amicon); following adsorption of the polynucleotide fraction onto the NACS column, gel matrix was washed out with 200 mM NaCl in 10 mM Tris buffer, pH 7.2, and then the polynucleotide fraction was eluted with 2 M NaCl in the same buffer.

Nucleotides. Nonradioactive CTP and GTP, HPLC-purified grade, were obtained from ICN. [5-^{3}H]CTP was from ICN or New England Nuclear. [γ-^{32}P]GTP, HPLC-purified grade, was from New England Nuclear; less purified material caused severe labeling artifacts in the procedure for measuring initiation.

Z-Inducing Cations. $[Co(NH_3)_6]Cl_3$ was obtained from Eastman Kodak; its concentration was measured by visible absorbance with use of ϵ_{473} = 56.2 (Widom & Baldwin, 1980) and by atomic absorption. $MgCl_2$ concentrations were checked by atomic absorption.

Measurement of Chain Initiation and Elongation. Initiation and elongation were measured simultaneously by incorporation of [γ-^{32}P]GTP and [^{3}H]CTP, respectively, at 37 °C. Reaction mixtures contained 20 nM RNAP holoenzyme, 20 μM polynucleotide, 25 μM each CTP and GTP, 500 μM Mg^{2+}, 50 mM NaCl, 5 mM Tris buffer, pH 8, and 100 μM DTT. [γ-^{32}P]GTP was used at 15000 Ci/mol and [^{3}H]CTP at 500 Ci/mol. To delay onset of any B to Z conversion at 500 μM Mg^{2+}, the Mg^{2+} was added to the reaction mixture immediately before the RNAP. The degree of B to Z conversion, checked independently by circular dichroism (see below), was < 10% by the last time point used, 6 min. Aliquots (100 μL) of the reaction mixtures were stopped by adding EDTA and KCl to 50 and 25 mM, respectively, and the excess of unincorporated nucleotide was removed by gel filtration (see Springgate and Loeb (1975)) on fresh columns of AcA-202 (IBF; nominal exclusion ~22000 daltons; ~10 × 200 mm). Fractions were collected until NTPs began to elute from the column and counted with addition of aqueous-miscible scintillant (Aquasure, New England Nuclear) in a Beckman 5800 scintillation counter. One peak representing RNA product emerges free from, but closely followed by, a large peak containing the excess NTPs. Due to the large excess of unincorporated over incorporated [γ-^{32}P]GTP, conventional acid precipitation does not adequately resolve the incorporated nucleotide, and it becomes necessary to use fresh gel filtration columns to reduce the background sufficiently. Incorporated nucleotide above background ranged from ~3- to ~100-fold for [γ-^{32}P]GTP and from ~6- to ~500-fold for [^{3}H]CTP, for the lowest to the highest values shown in Figure 2.

Chain-Length Distribution of RNA Products by Electrophoresis. (1) Short-Chain Products. RNA synthesis was conducted as described above, with 12000 Ci/mol [^{3}H]CTP. The 50-μL reactions were stopped by the addition of 500 μM EDTA and then urea (ultrapure grade, ICN) added to 8 M. These samples were electrophoresed at 10 V/cm on a 12-cm-long 20% polyacrylamide gel containing 8 M urea, in TBE buffer (Maniatis et al., 1982). Electrophoresis was continued until a bromphenol blue marker migrated about half the length of the gel. Lanes were cut serially into 4-mm sections, which were soaked overnight in a quaternary amine/toluene solution (NCS, Amersham, or Protosol, New England Nuclear); then, organic solvent based scintillant (Econofluor, New England

Nuclear) was added and the fractions were counted in a Beckman 5800 scintillation counter.

(*2*) *Longer Chain Products.* RNA synthesis was conducted as described above, with 12000 Ci/mol [^{3}H]CTP. The 100-μL reactions were stopped by the addition of 1 volume of buffer-washed phenol (BRL, ultrapure grade). After phenol extraction, product plus template was precipitated by ethanol, dried in vacuo, glyoxylated according to Charmichael and McMaster (1980), and then subjected to electrophoresis at 10 V/cm on a 12-cm-long 1.5% agarose gel, in 10 mM phosphate buffer. Mixtures of restriction fragments from ϕX- and λ-DNA (BRL) were similarly glyoxylated and used as chain-length markers; their positions in the gel were visualized by acridine orange staining after electrophoresis (Carmichael & McMaster, 1980). Electrophoresis was continued until a bromphenol blue marker migrated the full length of the gel. Lanes for the reaction mixtures were cut serially into 2-mm sections, which were extracted and counted as described above. A uniform chain-length scale was calculated from the positions of the restriction fragments, from 301 to 9400 nucleotide residues. At the low end of the scale—toward the bottom of the gel—a larger, practically invariant peak occurred in the distribution of ^{3}H label, which we assign to residual [^{3}H]CTP. It was centered at an apparent position of ~20 residues. The distribution of product was then calculated by subtraction of this component from the total distribution of label under the assumptions that it is symmetrical and that its maximum does not overlap the remaining distribution. After this subtraction was made, one more component emerged distinct from the rest of the distribution, centered at an apparent position of 50–100 residues; the distributions for this component and for the bulk of the product were calculated by subtraction from the total distribution of product in the same way.

Measurement of Free and Template-Bound RNAP by Sedimentation. Free and template-bound RNAP was determined from sedimentation boundary patterns (see Rezvin and Woychik (1981)), measured with a Beckman Model E analytical ultracentrifuge equipped with ultraviolet photoelectric scanning optics. Absorbance was recorded at 230 nm in order to obtain a sufficiently large signal for free and bound protein at the total protein concentrations used.

Measurement of Template Bound to RNAP by Filter Retention. The amount of poly(dGdm^{5}C)·poly(dGdm^{5}C) bound to RNAP was measured by retention, at 37 °C, on Millipore HA (0.45-μm pore size) filters of ^{32}P-end-labeled polynucleotide, according to the procedure of Hinkle and Chamberlin (1972).

Circular Dichroism. Circular dichroism measurements were performed at 37 °C with a Jasco J-500C spectropolarimeter, coupled to a Tektronix 4052A computer that drove the wavelength stepping motor and collected digitized ellipticity data. For spectra, ellipticities were collected at 2.5 nm-intervals and the digitized base lines were subtracted. For kinetic studies, ellipticities were collected sequentially at four successive wavelengths together with the elapsed time and the base-line values were subtracted. In experiments in which RNAP was added to the Z form of the polynucleotides, the CD spectrum was sampled during a 15-min period to ascertain that the spectrum had stabilized; up to about 15 min was required for the spectrum to stabilize at new values. To conserve materials, CD was measured for ~1.5-mL solutions in narrow rectangular cuvettes. RNAP was added in a relatively small volume of concentrated stock solution so as to minimize changes in the concentrations of the other components. The RNAP stock solution contained about 20 μM RNAP, in 10 mM Tris buffer, pH 8/5% glycerol/100 μM EDTA/100 μM DTT/100 mM NaCl (the preparation/storage medium for the enzyme, with NaCl decreased to 100 mM). Addition of RNAP in this manner to the 100 nM range increased the NaCl concentration in the order of 1%; such an increase in NaCl by itself had no measurable effect on the CD spectrum.

Results

(*A*) *Effects of Template Conformation on Binding, Chain Initiation, and Elongation. Experimental Definition of Conformers and Their Stability.* B and Z conformations were experimentally defined by the near-UV circular dichroism spectrum obtained under the conditions of transcription. This criterion as well as the B–Z transitions with respect to the concentration of the Z-inducing cations, Mg^{2+} and $[Co(NH_3)_6]^{3+}$, is presented in detail in part B; for measurements of transcription, the CD spectrum of the reaction mixtures, including nucleotide triphosphates and enzyme, was monitored. Divalent metal ions that support RNAP catalytic activity (Mg^{2+} in the present work) induce Z conformation in poly(dGdm^{5}C)·poly(dGdm^{5}C). To facilitate comparison of template conformation and transcription reactions, we chose a Mg^{2+} concentration at which chain initiation and elongation can be conveniently measured in the time during which 0–10% conversion of poly(dGdm^{5}C)·poly(dGdm^{5}C) from B to Z takes place but that is high enough to convert the polynucleotide approximately fully to the Z form at equilibrium. For the copolymer used in this work, this Mg^{2+} concentration[2] was 500 μM. The initial, nonequilibrium condition was used for the B control. The latter equilibrium condition is termed "moderately" stabilizing in this paper and corresponds to the phase boundary between mixtures of B and Z conformers, and an all-Z conformer (see Shin et al. (1988)). A third condition, "strongly" stabilizing the Z form, was provided by 10 μM $[Co(NH_3)_6]^{3+}$ (plus 500 μM Mg^{2+}), which is well beyond this phase boundary for the poly(dGdm^{5}C)·poly(dGdm^{5}C)/$[Co(NH_3)_6]^{3+}$ system but does not inhibit RNAP activity with templates that do not form Z (Butzow et al., 1984).

Effects of Z-Conformation Stability on Chain Initiation and Elongation. Measurement of the number of new product RNA chains formed by RNAP on poly(dGdm^{5}C)·poly(dGdm^{5}C) indicates (Figure 1a) that chain initiation is inhibited on the Z conformer as compared with the B. Elongation is also affected by conversion of the template from the B to the Z conformation. Two kinds of measurement were conducted to explore and correlate these effects: a time study of nucleotide incorporation into RNA product and a time study of product chain length.

(1) *Nucleotide Incorporation.* Parts a and b of Figure 1 show the time courses of chain initiation, measured by incorporation of [^{32}P]pppG into new 5′ chain ends, and of elongation measured simultaneously by incorporation of nucleotide triphosphates (as [^{3}H]CTP) into internal positions of the product. Both chain initiation and elongation are inhibited when the Z form is moderately stabilized over the B (500 μM Mg^{2+}). Strongly stabilizing the Z conformation with 10 μM $[Co(NH_3)_6]^{3+}$ leads to greater reduction in both chain initi-

[2] The concentration ranges of Mg^{2+} and $[Co(NH_3)_6]^{3+}$ for B–Z transition found in this work are somewhat lower than those found in our earlier work (Butzow et al., 1984). In particular, 500 μM Mg^{2+}, which here moderately stabilizes poly(dGdm^{5}C)·poly(dGdm^{5}C) in the Z form, in the earlier work is still within the all-B range. We do not know the cause of this difference and assume an artifact due to an unknown contaminant in the polynucleotide preparations used in the earlier work.

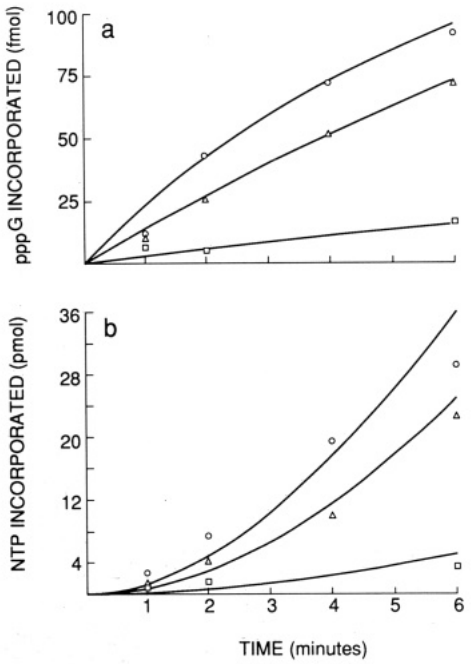

FIGURE 1: Measurement of chain initiation by incorporation of [^{32}P]pppG as the 5′ residues in the RNA product (panel a), and of elongation by incorporation of CTP + GTP into internal positions (detected by [^{3}H]CTP, panel b), with poly(dGdm^{5}C)·poly(dGdm^{5}C) in B or Z conformation as template: B form (○); Z form moderately stabilized by 500 μM Mg^{2+} (△); Z form strongly stabilized by 10 μM $[Co(NH_3)_6]^{3+}$ plus 500 μM Mg^{2+} (□). The data points (○, △, □) are the experimental measurements. The curves in panel a are fit to the pppG incorporation data by a single exponential. The curves in panel b are calculated by integrating the curves of panel a from time zero to current time and multiplying by a uniform chain growth rate selected at 110 nucleotides/min. Each reaction contains 20 nM holoenzyme, 20 μM (res) polynucleotide, 25 μM CTP and GTP, 50 mM NaCl, 5 mM Tris buffer, pH 8.0, and 100 μM DTT. Reaction temperature was 37 °C. Polynucleotide was sized to >1300 bp, to suppress template chain extension.

ation and elongation.[3] Actually, there appear to be parallel decreases in chain initiation and elongation as the stabilization of Z is increased. To examine the possible correlation between elongation and initiation, we calculated the time courses of NTP incorporation into internal positions from the time courses of pppG incorporation into new chain ends, under the assumptions that each new chain is elongated continuously at a uniform rate (the "chain growth rate") and that this rate is the same on B and Z templates. These assumptions appear to be reasonable on the basis of the time courses generated and on independent measurement of product chain lengths.

The experimental pppG incorporation data (points in Figure 1a) are fit by single exponentials (curves in Figure 1a). The fitted exponential curves are integrated from time zero to current time and then multiplied by a chain growth rate selected at 110 nucleotides/min. The resulting calculated curves (Figure 1b) fit the experimental elongation data points fairly well for all three cases, indicating that elongation may be limited by chain initiation and, therefore, that chain initiation, or a previous step, may be the limiting source of the inhibition of transcription on Z. The chain growth rate predicted by the elongation time courses, ~110 nucleotides/min, is somewhat

[3] The degree of inhibition of elongation found here with strongly stabilized Z conformer (Figure 1b) is considerably greater than what we observed earlier (Butzow et al., 1984). We attribute the larger residual activity found in the earlier work to chain extension on short DNA chains present in the copolymer preparations, which were not fractionated to eliminate short chains as were those used in the present transcription studies.

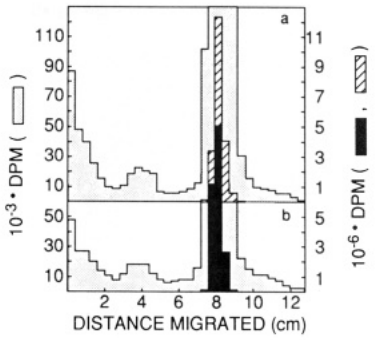

FIGURE 2: Distribution of ^{3}H-labeled RNA products synthesized in 4 min by RNAP on poly(dGdm^{5}C)·poly(dGdm^{5}C), and excess [^{3}H]CTP substrate, by gel electrophoresis in strongly sieving medium under denaturing conditions (20% polyacrylamide, 8 M urea): (a) template in the B form; (b) template in the Z form strongly stabilized by 10 μM $[Co(NH_3)_6]^{3+}$ plus 500 μM Mg^{2+}. The large peak centered about fraction 20 is unincorporated [^{3}H]CTP. Each reaction contains 20 nM holoenzyme, 20 μM (res) polynucleotide, 25 μM CTP and GTP, 500 μM Mg^{2+}, 50 mM NaCl, 5 mM Tris buffer, pH 8, and 100 μM DTT. Reaction temperature was 37 °C.

lower than the one estimated directly from the product chain lengths (~160/min, Figure 3). This difference could possibly be accounted for by some early termination and reinitiation, which would produce more new ends than expected from the bulk of internal NTP incorporation.

Study of the chain length of the product RNA provides more insights into the correlation between chain initiation and elongation.

(2) *Chain-Length Distribution of RNA Products.* In evaluating the differences in transcription shown in Figure 1, the possibility of greater production of abortive, short-chain RNA on the Z conformer needs to be considered. If there were more abortive synthesis on Z but the short-chain products were not counted in the protocol for Figure 1, the total synthesis (in long and short chains combined) could be much more similar for B and Z than Figure 1 indicates. To test this possibility, we analyzed the chain-length distribution down to the level of very short-chain products, by gel electrophoresis of the entire reaction mixture on strongly sieving polyacrylamide gel under denaturing conditions. The distribution of label after reaction, both for strongly stabilized Z-form template and B-form, is resolved (Figure 2) into long-chain product (at the top of the gel), a smaller amount of shorter chain product, and excess [^{3}H]CTP substrate. The shorter chain portion, which we assign to abortive product, is present in somewhat smaller amounts in the Z case. Thus, the possibility that the B–Z differences shown in Figure 1 are being influenced by a larger amount of abortive transcription on Z appears to be ruled out.

A detailed analysis of the longer chain-length products was carried out by use of electrophoresis through a weaker gel, after removal of enzyme and excess unincorporated substrate, and glyoxylation to effect strand separation and conversion to random chain conformation of DNA template and RNA product. The distribution of the labeled product found at successive times of reaction is shown in Figure 3 for template in the B form or moderately or strongly stabilized in the Z form. All product distributions, except the earliest time point for the low Mg^{2+}-stabilized Z case, show the presence of relatively short chain-length product as a subdistribution (dotted curves), centered at an apparent chain length between 50 and 100 residues, that stands out clearly in the total product distribution. This subdistribution likely represents the same short-chain-length product resolved on strongly sieving gel (note that the chain-length scale will not be accurate at its low

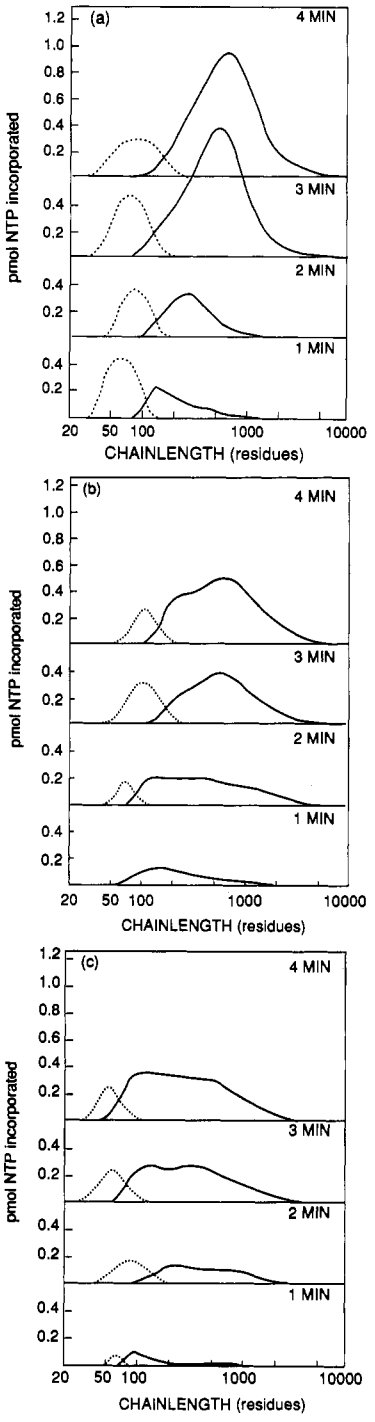

FIGURE 3: Chain-length distributions after different times of reaction, determined by gel electrophoresis, of RNA products synthesized by RNAP on poly(dGdm^{5}C)·poly(dGdm^{5}C) in (a) the B form, (b) the Z form moderately stabilized by 500 μM Mg^{2+}, and (c) the Z form strongly stabilized by 10 μM $[Co(NH_3)_6]^{3+}$ plus 500 μM Mg^{2+}. The RNA products are detected by the ^{3}H label incorporated from [^{3}H]CTP, and the distribution represents nucleic acid that has been denatured by glyoxylation. The chain-length scale is calculated from the positions of glyoxylated restriction fragment markers (from 301 to 9400 nucleotides long); electrophoretic migration is from right to left. In each panel for a reaction time, the product distribution is shown decomposed into two curves: a solid curve representing the bulk of the product and a dotted curve tentatively assigned to abortive product. The solid and dotted curves are calculated from the total distribution of ^{3}H label as explained under Experimental Procedures. *Each reaction* contains 20 nM holoenzyme, 20 μM (res) polynucleotide, 25 μM CTP and GTP, 500 μM Mg^{2+}, 50 mM NaCl, 5 mM Tris buffer, pH 8.0, and 100 μM DTT. Reaction temperature was 37 °C. Poly(dGdm^{5}C)·poly(dGdm^{5}C) was sized to >1300 bp, to suppress template chain extension.

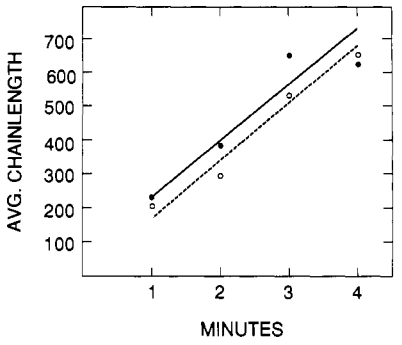

FIGURE 4: Centroidal positions of the polymeric portion of the RNA product distributions in Figure 3 (i.e., the distributions denoted by solid curves in Figure 3): product synthesized on poly(dGdm^{5}C)·poly(dGdm^{5}C) in the B form (○); product synthesized on poly(dGdm^{5}C)·poly(dGdm^{5}C) moderately stabilized in the Z form by 500 μM Mg^{2+} (●).

end since the shortest marker visualized has a chain length of 301 residues), and it is present in a smaller amount when the template is Z. The remainder of the label in the product distributions from the B and moderately stabilized Z template (Figure 3a,b; solid curves) shows a fairly consistent drift toward longer chain-lengths with time of reaction. A plot of the centroidal position of this portion, as a measure of polymeric product, against reaction time (Figure 4) indicates that the average chain growth rate for the polymeric RNA product is nearly the same, ~160 nucleotides/min, whether the template is in the B form or is moderately stabilized in the Z form.

In general, this longer chain-length region of the distribution of product made on moderately stabilized Z (Figure 3b) is broader than that of product made on the B form (Figure 3a), while that of product made on strongly stabilized Z (Figure 3c) is much broader. A bulge develops in the 100–200-residue range in the distributions for both Z cases. When the Z conformer is moderately stabilized, the bulge appears as a small shoulder on the main distribution. When Z is strongly stabilized, this bulge takes on a substantial portion of the distribution, although a fairly continuous shift toward a longer chain length still occurs in the remainder of the distribution (above ~200 residues). The existence of this bulge indicates that some inhibition of elongation occurs independently of the inhibition of chain initiation. It does not likely represent template chain extension because, while it definitely appears to be polymeric, it develops in a relatively short chain-length region of the distribution, well below the lower end of the template chain-length distribution.

Binding of RNAP to B and Z Forms. Since the transcriptional activity of RNAP appeared to be inhibited on the Z conformer at the chain initiation stage, it was appropriate to examine the binding characteristics of RNAP with both B and Z conformers. Three kinds of measurements were carried out. The total amounts of free and bound RNAP were measured by sedimentation velocity separation. The overall amount of polynucleotide bound to enzyme and the saturation of this binding with respect to enzyme concentration were measured by filter retention of the complexes. Exchange of poly(dGdm^{5}C)·poly(dGdm^{5}C) out of its complexes with RNAP was also measured by filter retention. The Z form of poly(dGdm^{5}C)·poly(dGdm^{5}C) was produced and kept under conditions in which it is very stable, namely 10 μM $[Co(NH_3)_6]^{3+}$, so that there would be little chance of conformational perturbation when a large excess of RNAP is added (see part B).

The RNAP–polynucleotide complexes were sedimented

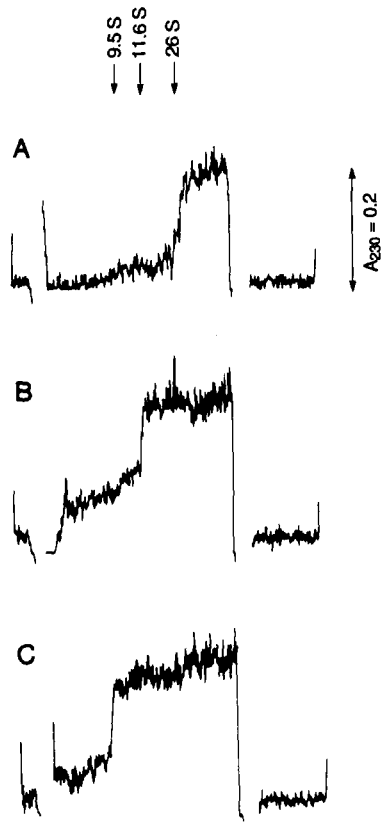

FIGURE 5: Sedimentation velocity measurement at 37 °C of the binding of RNAP holoenzyme to poly(dGdm^{5}C)·poly(dGdm^{5}C) in the B form and strongly stabilized in the Z form by $[Co(NH_3)_6]^{3+}$. Each system contains 50 mM NaCl and 5 mM Tris buffer, pH 8.0. Polynucleotide was sized to >1300 bp. The figure shows the 230-nm absorbance traces from centripetal (left) to the centrifugal positions in the sample after about the same time of sedimentation. The arrows over the traces mark the boundary positions, with the sedimentation rates found, for holoenzyme alone (9.5 S), the holoenzyme–B complex (26 S), and the holoenzyme–Z complex (11.6 S). No uncomplexed polynucleotide is detected; the polynucleotide used sediments in the range of 6 S in both B and Z forms. (A) 20 μM (res) polynucleotide in the B form with RNAP holoenzyme added to 100 nM: ~10% of the RNAP input (compare panel C) is free. (B) 20 μM (res) polynucleotide strongly stabilized in the Z form by 10 μM $[Co(NH_3)_6]^{3+}$ with RNAP holoenzyme added to 100 nM: ~20% of the RNAP input (compare panel C) is free. (C) 100 nM RNAP holoenzyme alone.

through the solution of free enzyme, which itself sediments more slowly, so that the complexes remained in equilibrium with the free enzyme. Sedimentation patterns for the RNAP–polynucleotide complexes and free RNAP, found at an RNAP:polynucleotide ratio close to saturation with respect to enzyme, are shown in Figure 5. No free polynucleotide is detected. The RNAP–polynucleotide complexes produced with the B form (panel A) sediment considerably more rapidly than either the free RNAP (panel C) or free polynucleotide; the relative height of the RNAP boundary (at ~9.5 S) indicates ~10% free enzyme. RNAP–polynucleotide complexes are also detected when the poly(dGdm^{5}C)·poly(dGdm^{5}C) is strongly stabilized in the Z form (panel B). The RNAP–Z complexes still sediment more rapidly than either free enzyme or polynucleotide but less rapidly than when the polynucleotide is in the B form. The relative height of the RNAP boundary indicates ~20% free enzyme for the Z case. Thus, strongly stabilized Z as well as B appears to bind considerable amounts of RNAP, with the Z binding less.

The overall amount of poly(dGdm^{5}C)·poly(dGdm^{5}C) bound to RNAP was examined at increasing RNAP concentrations (Figure 6), with polynucleotide in the B form or strongly stabilized in the Z form. Saturation appears to take place over

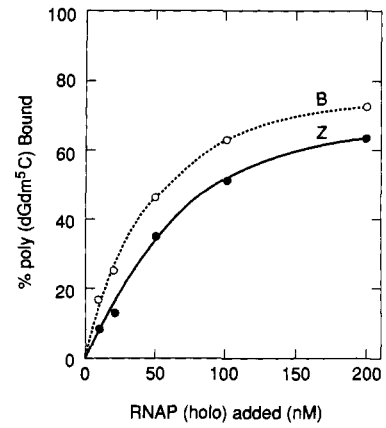

FIGURE 6: Saturation curves for binding to RNAP holoenzyme of poly(dGdm^{5}C)·poly(dGdm^{5}C) in the B form (---) and strongly stabilized in the Z form (—) by 10 μM $[Co(NH_3)_6]^{3+}$, measured by nitrocellulose filter retention of ^{32}P-end-labeled poly(dGdm^{5}C)·poly(dGdm^{5}C), at 37 °C. The initial poly(dGdm^{5}C)·poly(dGdm^{5}C) concentration is 20 μM (res). Each system contains 50 mM NaCl and 5 mM Tris buffer, pH 8.0. Polynucleotide was sized to >1300 bp.

a similar range of RNAP concentrations for both B and Z conformers. The fraction of polynucleotide bound at apparent saturation is substantially larger for the B as compared with the Z form, but the value is not several times greater for B than for Z. This interpretation of the saturation curves appears to be reasonable in view of the finding (Figure 5) that the amount of RNAP bound to the B and Z conformers is not greatly different.

Since measurements of the total amount of RNAP bound to poly(dGdm^{5}C)·poly(dGdm^{5}C) and of the overall binding of poly(dGdm^{5}C)·poly(dGdm^{5}C) bound to RNAP did not detect large differences with respect to the polynucleotide conformation, a study of polynucleotide exchange on RNAP was carried out. Poly(dGdm^{5}C)·poly(dGdm^{5}C) in the B form or strongly stabilized in the Z form was allowed to equilibrate with RNAP at a ratio of enzyme to poly(dGdm^{5}C)·poly(dGdm^{5}C) that provides roughly half-saturation (see Figure 6). Then, an excess of poly(dAdT)·poly(dAdT) was added and the amount of poly(dGdm^{5}C)·poly(dGdm^{5}C) remaining bound to the RNAP at various times after addition of the competing polynucleotide was measured by nitrocellulose filter retention. (Note that poly(dAdT)·poly(dAdT) remains in the B form when the $[Co(NH_3)_6]^{3+}$ concentration is raised from 0 to 10 μM.) The time course of release (Figure 7) shows that Z-form poly(dGdm^{5}C)·poly(dGdm^{5}C) is much more readily displaced from RNAP than is B-form poly(dGdm^{5}C)·poly(dGdm^{5}C). Therefore, while RNAP binds both B and Z forms of poly(dGdm^{5}C)·poly(dGdm^{5}C), it binds much less tightly to the Z form.

(B) Effects of RNAP Binding on Template Conformation. RNAP Perturbs the Z Conformation. When relatively high concentrations of RNAP are added to poly(dGdm^{5}C)·poly(dGdm^{5}C) induced into the Z conformation with either Mg^{2+} or $[Co(NH_3)_6]^{3+}$ or to poly(dGdC)·poly(dGdC) induced into the Z conformation with $[Co(NH_3)_6]^{3+}$, changes occur in the near-ultraviolet circular dichroism (CD) spectrum of the polynucleotide that are consistent with partial conversion of Z to B. Poly(dGdm^{5}C)·poly(dGdm^{5}C) is simpler to study since it is induced into the Z conformation at much lower concentrations of Mg^{2+} or $[Co(NH_3)_6]^{3+}$ than is poly(dGdC)·poly(dGdC) and since the unmethylated copolymer may undergo $[Co(NH_3)_6]^{3+}$-induced sequential conversion to A-like and ψ conformational states (Shin et al., 1988). Under the conditions used for the present studies, we have not detected any such

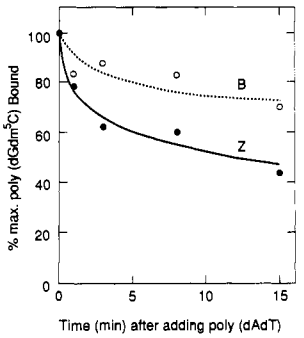

FIGURE 7: Time course of release from RNAP holoenzyme of poly(dGdm^{5}C)·poly(dGdm^{5}C) in the B form (---), and strongly stabilized in the Z form (—) by 10 μM $[Co(NH_3)_6]^{3+}$, after addition of excess poly(dAdT)·poly(dAdT) as competing polynucleotide, measured by nitrocellulose filter retention of ^{32}P-end-labeled poly(dGdm^{5}C)·poly(dGdm^{5}C) at 37 °C. The initial poly(dGdm^{5}C)·poly(dGdm^{5}C) concentration is 20 μM (res); the initial RNAP concentration is 50 nM, to correspond to ~50% of the saturation value (see Figure 6); poly(dAdT)·poly(dAdT) is added to 250 μM (res). Each system contains 50 mM NaCl and 5 mM Tris buffer, pH 8.0. Poly(dGdm^{5}C)·poly(dGdm^{5}C) was sized to >1300 bp.

conformational changes beyond the Z state for the methylated copolymer.

Figure 8a shows the effect on the CD spectrum of adding RNAP holoenzyme to poly(dGdm^{5}C)·poly(dGdm^{5}C) moderately stabilized in the Z form with Mg^{2+}. The Mg^{2+} concentration used initially to convert the polynucleotide from B to Z is only high enough so that at equilibrium most of the polynucleotide is in the Z form. Holoenzyme at 100 or 200 nM is added to 20 μM (res) polynucleotide (1 RNAP:100 bp or 1 RNAP:50 bp, respectively). After addition of the RNAP, the starting CD spectrum changes to spectra that appear to represent mixtures of B and Z, and, as shown in Figure 8a, are mathematically fit very well as linear combinations of experimental spectra for 100% B (B std) and the maximum Z characteristics obtained (Z std). The higher the RNAP:polynucleotide ratio, the greater the apparent conversion of Z to B. The CD spectrum continues to change over a period of about 15 min after the addition of RNAP. The same kind of spectral effects are produced when RNAP is added to poly(dGdm^{5}C)·poly(dGdm^{5}C) moderately stabilized in the Z form with $[Co(NH_3)_6]^{3+}$ (2.5 μM, Figure S1 in supplementary material) or to the unmethylated copolymer poly(dGdC)·poly(dGdC) moderately stabilized in the Z form with $[Co(NH_3)_6]^{3+}$ (Figure S2 in supplementary material). In all these cases the polynucleotide spectra are very well fit as mixtures of B and Z. The generality of the perturbing effect of RNAP on the CD spectrum indicates strongly that the result of RNAP interaction with the Z form really has been partial conversion from Z to B.

Other possible origins of these CD spectral changes might include formation of a single-stranded structure or of another duplex conformation such as one of the A type. When poly(dGdm^{5}C)·poly(dGdm^{5}C) is heated above the temperature range for the double-stranded B to single-stranded transition, the CD spectrum (Figure 8b) does not resemble the B type sufficiently to fit the large-scale perturbations found with RNAP, although small fractions of such a spectral form could be accommodated along with the typical B type. Poly(dGdC)·poly(dGdC) can be converted from the Z conformation into a so-called U conformation in the presence of $[Co(NH_3)_6]^{3+}$, but the CD spectrum of U is very unlike that of the starting B form (Shin et al., 1988).

We limited our further examination of the perturbation of the Z form by RNAP to the poly(dGdm^{5}C)·poly(dGdm^{5}C)/$[Co(NH_3)_6]^{3+}$ or Mg^{2+} systems.

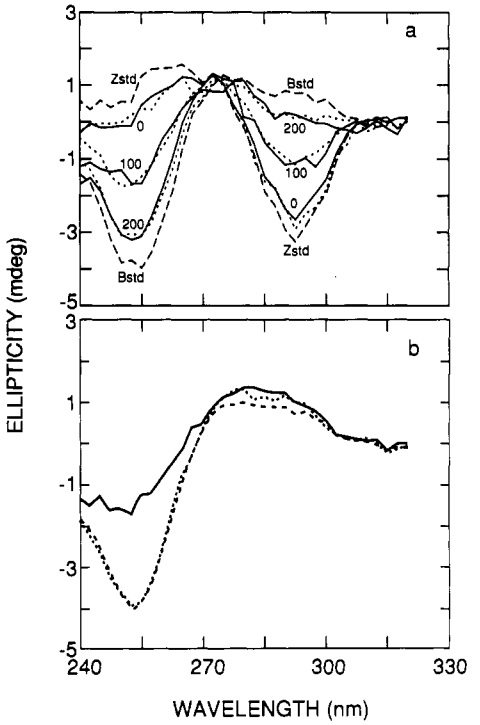

FIGURE 8: (a) Perturbation of the polynucleotide CD spectrum produced on addition of 100 or 200 nM RNAP holoenzyme to 20 μM (res) poly(dGdm^{5}C)·poly(dGdm^{5}C) moderately stabilized in the Z conformation with 450 μM Mg^{2+}, in 50 mM NaCl/5 mM Tris buffer, pH 8.0, 37 °C. Each experimental spectrum (—) is identified by the concentration of RNAP added (nanomolar), and is accompanied by a fitted spectrum (···) representing the best fit to the experimental of linear combinations of the reference B and Z spectra (---), B std and Z std. B std refers to the starting B form; Z std refers to the maximum Z CD effect obtained with Mg^{2+}. The fitted curves indicate 85% Z + 15% B for no RNAP, 48% Z + 52% B for 100 nM RNAP, and 17% Z + 83% B for 200 nM RNAP. b) CD spectrum of 20 μM (res) poly(dGdm^{5}C)·poly(dGdm^{5}C) in 100 μM Na_2EDTA, heated above the double-stranded B to single-stranded transition, at 60 °C (—); the original sample at 37 °C (---); and the sample heated to 60 °C and then cooled to 49 °C (···). As judged by CD, the sample melted above 49 °C and below 54 °C.

RNAP Shifts the B–Z Transition. We traced the effect of RNAP on the Z form of poly(dGdm^{5}C)·poly(dGdm^{5}C) in two ways. First, experiments of the type illustrated in Figure 8a were repeated at several different concentrations of Mg^{2+} or of $[Co(NH_3)_6]^{3+}$ that spanned the B–Z transition regions for the two Z-inducing cations. After equilibration of each sample at a particular Mg^{2+} or $[Co(NH_3)_6]^{3+}$ concentration, the polynucleotide CD spectrum was measured before and after addition of holoenzyme to a ratio of 100 nM RNAP:20 μM (res) polynucleotide (1 RNAP:100 bp). The resulting CD spectra were analyzed as in Figure 8a for the relative contributions of the 100% B and 100% Z spectra. In each case the experimental spectrum was fit well in terms of mixtures of B and Z. The calculated fractions of Z before and after RNAP addition are plotted against the concentration of Z-inducing cation in Figure 9. With both Mg^{2+} and $[Co(NH_3)_6]^{3+}$, the interaction with RNAP is seen to shift the B–Z transition to a higher Z-inducing cation concentration.

We further examined the perturbing effect of RNAP on Z by making CD measurements for poly(dGdm^{5}C)·poly(dGdm^{5}C) initially induced nearly completely into the Z form at a single concentration of Mg^{2+} or of $[Co(NH_3)_6]^{3+}$, before and after the addition of various concentrations of RNAP. The results (Figure 10) indicate that a wide range of perturbation takes place as the ratio of RNAP to polynucleotide is changed.

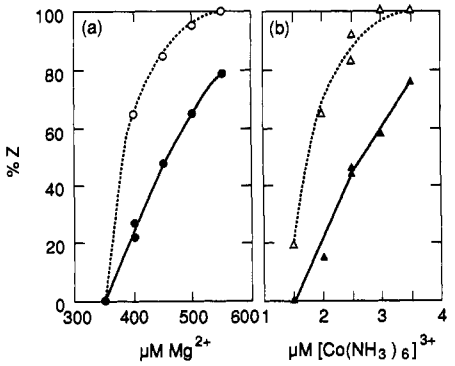

FIGURE 9: RNAP-induced apparent shift of the B–Z transition of poly(dGdm^{5}C)·poly(dGdm^{5}C) to higher concentrations of Z-stabilizing cation. Polynucleotide solutions (20 μM (res)) are equilibrated at 37 °C in 50 mM NaCl/5 mM Tris buffer, pH 8.0, with various concentrations of Z-inducing cation but without RNAP (---), and then RNAP holoenzyme is added to 100 nM (—). The percent of Z is calculated from CD spectra at 37 °C, as illustrated in Figure 8a: (a) Z stabilized by Mg^{2+}; (b) Z stabilized by $[Co(NH_3)_6]^{3+}$.

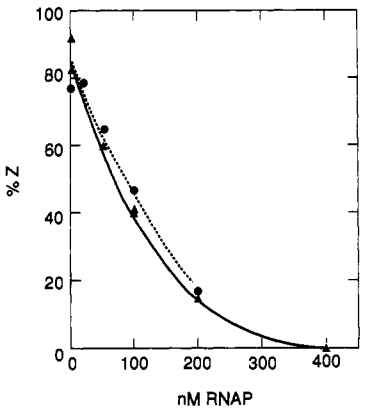

FIGURE 10: RNAP concentration dependence of the RNAP-induced apparent Z to B conversion, for poly(dGdm^{5}C)·poly(dGdm^{5}C). Polymer solutions (20 μM (res)) are equilibrated at 37 °C in 50 mM NaCl/5 mM Tris buffer, pH 8.0, plus either 2.5 μM $[Co(NH_3)_6]^{3+}$ (—) or 450 μM Mg^{2+} (---), then RNAP holoenzyme is added to the concentration shown. The percent of Z is calculated from CD spectra at 37 °C, as illustrated in Figure 8a.

At sufficiently high RNAP, apparently complete conversion of Z to B takes place. On the other hand, it appears that some conversion to B occurs at quite low RNAP levels. These results suggest that any amount of RNAP binding to the Z form could produce some degree of conversion to B.

While the studies shown in Figures 8–10 were made by use of polynucleotide samples not further purified to restrict their chain-length distribution, due to the amount of polynucleotide required, the same kind of effect of RNAP on Z occurs with a polynucleotide population from which relatively short chains have been removed (see Figure 12).

RNAP Inhibits B to Z Conversion. RNAP added to the B form of either poly(dGdm^{5}C)·poly(dGdm^{5}C) or poly(dGdC)·poly(dGdC) before addition of the Z-inducing cation lowers the rate at which the Z form is subsequently produced. Figure 11 shows the time course of the ellipticity for poly(dGdm^{5}C)·poly(dGdm^{5}C) and Mg^{2+} at characteristic wavelengths: in the 250-nm region, where the B form has a large trough, in the 290-nm region, where the Z form has a large trough, at the isodichroic wavelength (273 nm), and at a control wavelength (320 nm) where both B and Z have approximately zero ellipticity. The very slight change in the signal at the isodichroic wavelength for B and Z forms indicates that CD detects little besides these two conformational species. Addition of enzyme to a ratio of 100 nM RNAP:20

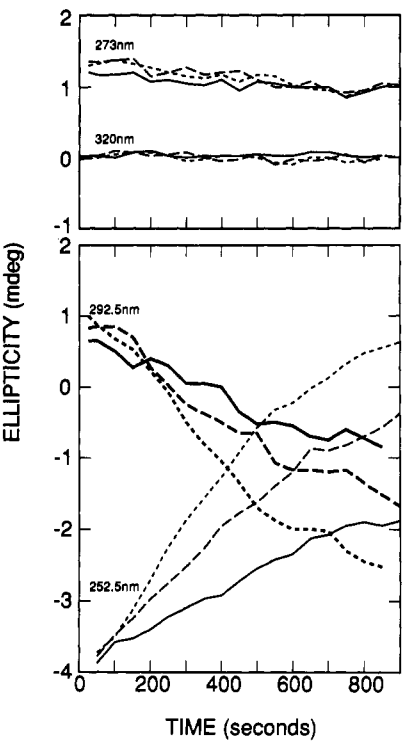

FIGURE 11: Inhibition of B to Z conversion of poly(dGdm^{5}C)·poly(dGdm^{5}C) by RNAP. The ellipticity is monitored at four wavelengths: at two prominent bands in the B and Z spectra (lower panel, 252.5 nm, lighter lines, and 292.5 nm, heavier lines), and (upper panel) at the isodichroic point (273 nm) and at a control wavelength outside the absorbance envelope (320 nm). 20 μM polynucleotide solutions are equilibrated, with or without the addition of RNAP holoenzyme, at 37 °C in 50 mM NaCl/5 mM Tris buffer, pH 8.0; then, 500 μM Mg^{2+} is added. Time courses are shown for the absence of RNAP (---), after addition of RNAP to 20 nM (--), and after addition of RNAP to 100 nM (—). Polynucleotide was sized to >1300 bp.

μM (res) polynucleotide (1 RNAP:100 bp) produces a large inhibition of B to Z conversion, whether Z is induced by Mg^{2+} or $[Co(NH_3)_6]^{3+}$. However, a substantial inhibition also occurs when enzyme is added to a ratio of 20 nM RNAP:20 μM (res) polynucleotide (1 RNAP:500 bp), while the effect of adding such a concentration of RNAP to polynucleotide already converted to Z is only just detectable (Figure 10). The effect of RNAP on the $[Co(NH_3)_6]^{3+}$-induced B–Z conversion of poly(dGdC)·poly(dGdC) was also studied (Figure S3 in supplementary material), and similar results were obtained. Since inhibition of B to Z conversion at RNAP:100 bp is readily detected with polynucleotide sized to remove shorter chains (Figure 11), possible interaction of RNAP with polynucleotide chain ends would not likely account for the phenomenon.

Specificity of RNAP in Perturbing Z and Inhibiting B to Z Conversion. If core polymerase (RNAP without the σ subunit) is used instead of the holoenzyme, both the perturbing effect on Z and the inhibitory effect on initial B to Z conversion are considerably reduced. Figure 12 shows that addition of core polymerase to a ratio of 100 nM core:20 μM (res) poly(dGdm^{5}C)·poly(dGdm^{5}C) (1 RNAP:100 bp) still causes some apparent Z to B conversion, but much less than that produced by the same amount of holoenzyme. Similarly, Figure 13 shows that some inhibition of B to Z conversion still occurs at 1 core:100 bp core enzyme, but the effect is only a fraction of that given by the same concentration of holoenzyme. Since core polymerase retains the capacity for general, nonspecific binding to DNA and its nonspecific binding can be stronger than that of holoenzyme (Hinkle & Chamberlin, 1973), there appears to be considerable specificity in the effects

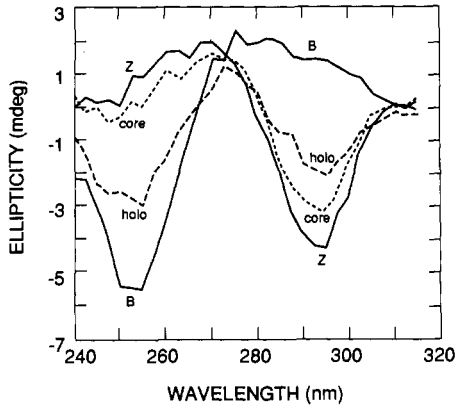

FIGURE 12: A lower degree of apparent Z to B conversion produced when RNAP core enzyme as compared with holoenzyme is added to 20 μM (res) poly(dGdm^{5}C)·poly(dGdm^{5}C) moderately stabilized in the Z conformation with 450 μM Mg^{2+}. Polynucleotide CD spectra marked core and holo were obtained after equilibration at 37 °C with Mg^{2+} to produce Z; then either core enzyme or holoenzyme was added to 100 nM. Polynucleotide CD spectra shown in solid lines and marked B and Z were obtained with the polynucleotide before and after equilibration with Mg^{2+} (but without RNAP), respectively. Polynucleotide was sized to >1300 bp.

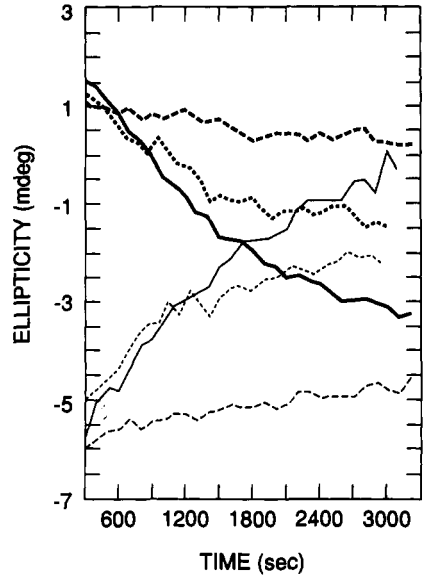

FIGURE 13: Less inhibition of B to Z conversion produced by RNAP core enzyme as compared with holoenzyme. Time courses of conversion at 37 °C of 20 μM (res) poly(dGdm^{5}C)·poly(dGdm^{5}C) by 450 μM Mg^{2+} are shown in terms of the ellipticity at two prominent bands in the B and Z spectra (252.5 nm, lighter lines, and 292.5 nm, heavier lines) in the absence of RNAP (—) and after addition of either core (---) or holoenzyme (--) to 100 nM. Polynucleotide was sized to >1300 bp.

of holoenzyme on Z and on B to Z conversion.

Discussion

Effects of Template Conformation on Binding, Chain Initiation, and Elongation. The studies of binding, chain initiation, and elongation reported in this paper indicate that each of these stages in the action of *E. coli* RNA polymerase on its template may be affected when the template conformation is changed from B to left-handed Z.

Inhibition of chain initiation occurs already under conditions of moderate stabilization in the Z form, as measured by pppG incorporation into new RNA chains (Figure 1a). Elongation, as measured by NTP incorporation into internal positions of the product, is also inhibited (Figure 1b), and comparison of the time courses of chain initiation and elongation indicates that chain initiation may effectively limit subsequent elongation when the template is in the B or is moderately stabilized in the Z conformation. Thus, the observed inhibition of transcription on the Z form appears to stem from inhibition of chain initiation, either direct inhibition or inhibition due to interference at an earlier stage. This conclusion is reinforced by the finding that the average chain growth rate of the RNA product, measured from the change with time of the product chain length (Figure 3), is very similar whether the template is in the B or moderately stabilized in the Z form—even though accumulation of RNA product is substantially lowered on the Z form. If the template is strongly stabilized in the Z form, both chain initiation and overall elongation are inhibited more severely (Figure 1a,b), and there is still a rough correlation between NTP and pppG incorporation that suggests that chain initiation is controlling subsequent elongation.

The Z form does retain the ability to bind RNAP, at least nonspecifically, as judged by the measurements of total binding with poly(dGdm^{5}C)·poly(dGdm^{5}C) strongly stabilized in the Z conformation. Measurement of the saturation of the total binding of poly(dGdm^{5}C)·poly(dGdm^{5}C) with respect to RNAP concentration (Figure 6), and of the total amount of RNAP bound near saturation (Figure 5), leads to the conclusion that overall binding is moderately better when the polynucleotide is in the B form. The study of the release of the poly(dGdm^{5}C)·poly(dGdm^{5}C) bound, by competition with another polynucleotide, shows (Figure 7) that the Z conformer is much more readily released from the enzyme than the B conformer and, thus, that RNAP binds the B form much more tightly. The amount and rapidity of the release indicate that the initial complex of RNAP with Z conformer is not readily converted to a kinetically more stable complex—possibly one in which the template is locally unwound. Thus, RNAP can discriminate between B and Z conformations very early in the transcription pathway, already at the template-binding stage.

In considering the effect of Z conformation on template binding along with that on catalytic activity, we are implicitly assuming that the binding measured refers to catalytically competent RNAP. Catalytically incompetent RNAP may retain binding capacity (Hinkle et al., 1972). However, the assumption appears reasonable in the present work, since the RNAP preparations used contain 70–80% fully active molecules and since the binding measurements are performed at, or extend to, fairly high RNAP:template ratios.

While RNAP is sensitive to the Z conformation at the stages of binding and chain initiation, it also appears to be subject to additional inhibition at the elongation stage, as shown by the accumulation (Figure 3) of some relatively short-chain polymeric product when the template is moderately stabilized in Z and considerably more accumulation when strongly stabilized in Z. Since the product is polymeric, it likely represents early termination, but not abortive synthesis, or the result of blockage of elongation. The changes in the RNA product distribution with time (Figure 3) indicate, however, that RNAP engages in largely processive synthesis when the template is moderately stabilized in the Z conformation and that some processive synthesis continues even if strongly stabilized.

The present studies, therefore, indicate that the inhibition of RNAP action on templates in the Z conformation is due to very early events in the reaction cycle, even at the level of binding, although elongation may be affected independently. The finding by Job et al. (1988) that single-nucleotide addition to CpG primer by wheat germ RNA polymerase II, working on templates of the $[(dCdG)\cdot(dGdC)]_n$ type, is enhanced when the template is in the Z conformation likewise points to in-

terference at early stages in the reaction pathway.

If processive synthesis on the Z form is inhibited at the binding or chain initiation stage, how then is the residual processive synthesis that is observed to be explained? That is, what allows escape from this inhibition? Considering our finding that the RNAP perturbs the DNA conformation, shifting the B–Z equilibrium toward B, we are tempted to speculate that any processive transcription on Z involves RNAP-assisted, probably local, conformational change in the template to the B form. Such RNAP-assisted conformational change would depend on the local stability of the Z form. The occurrence of some initiation and elongation on template that is moderately stabilized in Z, but less when strongly stabilized in Z, could then be a result of local conversion from Z to B through template interaction with the enzyme.

Effects of RNAP Binding on Template Conformation. The conformational studies presented in this paper indicate that when *E. coli* RNA polymerase binds to DNA, the B conformation is stabilized vis-a-vis the Z conformation, thus shifting the B–Z equilibrium toward the B form. Using the alternating copolymer poly(dGdm^{5}C)·poly(dGdm^{5}C) as well as the unmethylated poly(dGdC)·poly(dGdC), we detect by circular dichroism two sorts of effects on the conformation of the polynucleotide when it is bound by the enzyme. If the polynucleotide is initially in the Z form and its stability in the Z form is not too high, RNAP binding leads to the apparent conversion of Z to B (Figures 8a, S1, and S2). The B–Z transition is shifted to higher metal ion inducer concentrations when RNAP is allowed to bind the DNA (Figure 9). If the polynucleotide is initially in the B form, RNAP binding leads to inhibition of conversion of B to Z when inducer is added (Figures 11 and S3). Both effects are increased in magnitude with the increasing ratio of RNAP to polynucleotide. Both effects are detected by CD down to fairly low RNAP:polynucleotide ratios. Perturbation of Z was detectable down to a practical limit of about 1 RNAP:500 bp (Figure 10); inhibition of B to Z conversion was still readily detectable at 1 RNAP:500 bp (Figure 11). Five hundred base pairs is in the order of 10 times the length of double-stranded DNA contacted by RNAP (see Siebenlist et al. (1980)). Finally, both of these effects appear to entail a considerable specificity in the interaction of RNAP with the DNA, since core enzyme ($\alpha_2\beta\beta'$) is much poorer than holoenzyme ($\alpha_2\beta\beta'\sigma$) in producing either of them (Figures 12 and 13). Possibly this specificity involves the σ subunit. Since the σ subunit is associated with specific binding and initiation, this result is clearly in line with our finding that the effect of Z conformation on transcription by RNAP can be predominantly at the stage of chain initiation or earlier steps.

The CD spectral data we have collected are entirely consistent with conversion of polynucleotide initially in the Z form to the B or a B-like form, although these data do not absolutely prove that the species produced is a B form. The conclusion that reconversion to B takes place is reinforced by the finding that RNAP apparently shifts the B–Z transition to higher concentrations of Z-inducing cations. The inhibition of the initial B to Z conversion by RNAP even more clearly points to the B form as being produced by binding of RNAP to the DNA.

The stabilization of the B conformer and destabilization of the Z indicated by the circular dichroic effects seem to be in accord with the demonstrated binding preference of RNAP for the B form over the Z (Figures 5–7), especially the much more ready release of the Z conformer from the enzyme. One can speculate, on the basis of the disparity in the ability of the B and Z conformers to bind RNAP, that any region in a polynucleotide chain globally in the Z conformation but able to undergo a local fluctuation to the B conformation would form a nucleus for preferential RNAP binding and Z to B conversion.

It may be appropriate to note here the effects on the B–Z equilibrium brought about by the binding of intercalating drugs such as ethidium, actinomycin D, and daunomycin to [(dCdG)·(dGdC)]$_n$-type polynucleotides. Both conversion of Z to B and inhibition of B to Z conversion have been noted (Mirau & Kearns, 1983; Chaires, 1983, 1985; Walker et al., 1985a,b). The binding of one molecule of intercalator can result in the conversion of several base pair residues from Z to B, indicating cooperative, long-range effects (Chaires, 1985; Walker et al., 1985a,b). Under some conditions, intercalator molecules cluster in B regions (Walker et al., 1985a,b; Chaires, 1986a,b). Of course, the interaction of RNAP with DNA and that of intercalating drugs with DNA may be expected to differ because of the intercalation alone and because the DNA binding site for RNAP is much larger than those for such drugs. Therefore, the mechanism by which RNAP affects the template conformation may or not be similar to that of these drugs.

The relationship between binding of RNAP to DNA and the RNAP-induced effects on the DNA conformation might, in principle, be complicated by the presence of inactive RNAP in the population of enzyme molecules. However, the RNAP preparations used contained 70–80% active molecules, and RNAP molecules that are catalytically inactive may still be active in binding (Hinkle et al., 1972). If they are not, the effects on template conformation would be due entirely to the active enzyme component. Comparison between RNAP binding and its conformational effects might also, in principle, be complicated by binding of RNAP to DNA chain ends or at single-strand cleavages or gaps in the body of the chain. But it is unlikely that RNAP binding to any such possible areas outside the body of the chain makes a substantial contribution to the conformational effects measured. Inhibition of B to Z conversion is seen with poly(dGdm^{5}C)·poly(dGdm^{5}C) fractionated to eliminate short chains; and conversion of Z to B of a similar degree is seen with unfractionated and fractionated copolymer. Moreover, we consider that binding of RNAP to a relatively small number of chain ends or nicked or gapped regions should be saturated at low RNAP concentrations, while experimentally we find that the conformational effects of RNAP binding clearly are increased by increasing the RNAP concentration over a broad range.

The effects on the B–Z equilibrium that occur on interaction of RNAP with DNA whose sequence can accommodate the Z conformation are clear indications that this enzyme has a strong bias for the B as opposed to the Z conformation in its interaction with templates. Differentiation between B and Z conformations could involve, besides helix handedness, the different topography of their helical grooves, differences in phosphate charge orientation, or even differences in the orientation of particular kinds of base residues (for a review on Z structure, see Rich et al. (1984)). Since histones (Russel et al., 1983) and basic, Z-specific binding proteins (Nordheim et al., 1982), as well as certain basic polypeptides (Russel et al., 1983), have been reported to influence the B–Z equilibrium, it is possible that the orientation of DNA phosphate groups is a determining factor in RNAP effects on DNA conformation.

Conclusions

The present studies, using as models the B and Z conformers

of poly(dGdm^{5}C)·poly(dGdm^{5}C), show that the secondary structure of a tract of DNA and the stability of that structure can critically affect the ability of the DNA to support the binding, RNA chain initiation, and processive synthetic activities of *E. coli* RNA polymerase. The effects of DNA conformation on the earliest stages of the reaction pathway, in particular, provide evidence that DNA secondary structure plays a critical role in the recognition by RNAP of regions of a template. Moreover, these studies show that the enzyme, in binding to template, restricts template conformation, bringing about a shift in DNA conformational equilibrium. The dependence of this restriction on the holoenzyme likewise points to early, specific recognition effects. All these considerations suggest that a B or B-like structure may be required, at least initially, for stable, specific RNAP–DNA complex formation. One result of such a requirement would be the destabilization of an alternative conformation, such as the Z form.

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Supplementary Material Available

Figures S1 and S2 presenting polynucleotide CD spectra showing the effect of RNAP addition on polynucleotide conformation for poly(dGdm^{5}C)·poly(dGdm^{5}C) and poly(dGdC)·poly(dGdC), respectively, initially stabilized in the Z conformation by $[Co(NH_3)_6]^{3+}$, and Figure S3 showing the effect of RNAP on the time course of the B to Z conversion of poly(dGdC)·poly(dGdC), the Z-inducing cation being $[Co(NH_3)_6]^{3+}$ (3 pages). Ordering information is given on any current masthead page.

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