0022-538X/78/0025-0305$02.00/0
JOURNAL OF VIROLOGY, Jan. 1978, p. 305-311

Vol. 25, No. 1
Printed in U.S.A.

Effect of Chemical Modification of Supercoiled Simian Virus 40 DNA on the Rate of In Vitro Transcription

PATRICIA HALE AND JACOB LEBOWITZ*

Department of Microbiology and Comprehensive Cancer Center, University of Alabama in Birmingham, Birmingham, Alabama 35294

Received for publication 21 June 1977

Superhelical simian virus 40 FI DNA could be modified with the single-strand-specific reagent *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium)ethylcarbodiimide (CMC). A limited reaction, of less than 2% of the base pairs, resulted in almost total inhibition of in vitro transcription by DNA-dependent RNA polymerase from *Escherichia coli*. This effect was shown to be due to DNA modification and not to inhibition of polymerase activity by the reagent. Inhibition of enzyme activity occurred if the contaminating reagent was not absorbed with another protein before polymerase addition. No inhibition was observed when DNA and polymerase were incubated together to allow the formation of pre-initiation complexes before CMC was added. Studies of template saturation with polymerase showed that the inhibition of transcription by DNA modification was due to a loss of binding ability of the enzyme to the reacted, supercoiled DNA when reaction times of less than 2 h were used.

Supercoiled (FI) DNA has been shown in several systems (2, 3, 6, 10, 18, 19, 22) and in simian virus 40 (SV40) (15; see accompanying paper [9]) to act as a more efficient template for RNA synthesis by DNA-dependent RNA polymerase than relaxed, or nonsupercoiled (FII), DNA. Unpaired regions in supercoiled DNA have been indicated by a number of different methods (12; 21 reviews literature through 1976). The greater template activity of supercoiled DNA suggests that these unpaired regions, produced by supercoiling, may be directly involved in the transcription process.

Reagents specific for unpaired bases in DNA allow for the exploration of the role of single-stranded regions in biological processes. *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium)ethylcarbodiimide-*p*-toluene sulfonate (CMC) is one such reagent. At neutral pH, CMC binds covalently to the imino (NH) sites of guanine and thymine (17). Since hydrogen bonding between base pairs must be interrupted to accomodate the bulky CMC reagent, it would be anticipated that reactivity should not occur without either transient distortion of the Watson-Crick structure or a preexisting disrupted secondary structure. Consequently, the reagent reacts readily with single-stranded DNA, whereas duplex DNA is essentially unmodified.

In the case of superhelical DNA, it has been previously shown that CMC reacts with SV40 FI DNA but not with nicked FII DNA (13). The sites of CMC reaction map in the endonuclease *Hin* II-III fragments (A, B, and G) (5) shown to contain at least five RNA initiation sites (14). Since these results also suggest the involvement of single-stranded regions in transcription, we initiated a study of the effect of CMC modification upon DNA template ability for RNA synthesis by *Escherichia coli* RNA polymerase.

Previous preliminary studies with superhelical SV40 and PM2 DNAs show a pronounced loss of transcription upon the initial reaction of FI DNA with CMC (6, 8). In this study, we utilize template saturation experiments to analyze which step in polymerase function is blocked by CMC. In addition, we have ruled out that inhibition results from a loss of enzyme activity due to CMC modification. Hence, the results firmly establish that very limited modification of SV40 DNA I is responsible for the loss of template function. The data will be compared to the previous study of the binding of *E. coli* RNA polymerase to SV40 DNA and other recent studies on the in vitro transcription of SV40 DNA (14).

MATERIALS AND METHODS

Preparation of SV40 DNA. CV-1 cells were grown in minimum essential medium (Eagle) with Hanks salts, 2 mM glutamine, and 10% fetal calf serum. Confluent monolayers were infected with SV40 virus (small-plaque strain, obtained from D. Nathans) at an input multiplicity of 5 to 20 PFU per cell in minimum essential medium, 2 mM glutamine, and 2% fetal calf serum. After a 36-h incubation at 37°C, super-

coiled SV40 DNA I was recovered according to the Hirt procedure (11) followed by ethanol precipitation, subsequent sedimentation to equilibrium in CsCl-ethidium bromide gradients, and storage in 6 mM Tris-hydrochloride (pH 7.4)–0.3 mM EDTA at −20°C.

Reaction of SV40 DNA with CMC. SV40 DNA I (100 μg/ml) was reacted with 500 mol of CMC per mol of DNA-phosphate in 10 mM $Na_2B_4O_7$, pH 8.0, at 37°C (13). After CMC reaction, samples were applied to a 10-ml CM Bio-Gel A column and eluted with 0.01 M KPO_4, pH 6.0, buffer and then dialyzed into DNA storage buffer containing 6 mM Tris-hydrochloride and 0.3 mM EDTA, pH 7.4. This column step results in ~95% removal of excess, unreacted CMC reagent. All CMC solutions are prepared the same day as they are used.

Preparation of polymerase. *E. coli* DNA-dependent RNA polymerase was obtained in one of four ways, (i) prepared according to a recent procedure (4), (ii) purchased from Grand Island Biological Co., (iii) as a gift from M. Flashner, or (iv) as a gift from R. Burgess. In all cases, the polymerase was stored in 10 mM Tris-hydrochloride–5 mM $MgCl_2$–1 M KCl–0.1 mM EDTA–10 mM β-mercaptoethanol–50% glycerol, pH 7.9, at −20°C. The amount of sigma in the holoenzyme preparations was determined by rifampin inhibition, according to the procedures of Mangel and Chamberlin (16). In all cases, sigma content was greater than or equal to 80%.

Transcription assay. The assay mixture consisted of 40 mM Tris-hydrochloride (pH 8.0), 8 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.8 mM K_2HPO_4, 100 mM KCl, 0.5 mg of bovine serum albumin per ml, and 2.5 mM each ATP, UTP, GTP, and CTP. [^{3}H]ATP was added to the triphosphates such that a 1:12.5 dilution of the triphosphates gave a specific activity of this compound between 8,000 and 12,000 cpm/nmol. Length of assay and DNA, RNA polymerase, and rifampin concentrations were as described in the figure legends. In all experiments, DNA and polymerase were incubated together for 10 min at 37°C before addition of triphosphates, except where indicated.

RESULTS

Transcription inhibition by CMC reaction with SV40 DNA I. The reaction of SV40 DNA with the water-soluble carbodiimide CMC has recently been characterized by a variety of procedures. By sedimentation velocity and buoyant density criteria, CMC has been found to react only with FI DNA (13). Buoyant density experiments have also shown that after a 24-h reaction, only 108 molecules of CMC are bound per molecule of SV40 DNA (representing 2% of the DNA, in terms of base pairs). The use of [^{14}C]CMC has allowed a quantitative analysis of the binding to individual *Hin* II-III restriction fragments. Only fragments A, B, and G (in the early region) react, and the analysis has shown that 2.24% of the base pairs are modified, in agreement with the buoyant density estimate (5). CMC reactivity has, consequently, been chosen in these studies as a sensitive probe to study the effect of limited reactivity at unpaired bases on transcription.

Figure 1 shows the effect of CMC reaction on transcription of FI SV40 DNA. In the presence of rifampin, >50% inhibition of transcription was observed after only 30 min of CMC reaction. This inhibition increased to 85% at 2 h of reaction and to 98% after 12 to 24 h of reaction of CMC with the DNA. As would be expected, similar results were observed with reacted SV40 DNA I in the absence of rifampin. None of the procedures previously used to detect CMC were sensitive enough to measure the CMC bound during such short reaction times as 2 h (5, 13).

Characterization of CMC effect on polymerase activity. To eliminate the possibility that the inhibition effect observed was due to contaminating CMC reagent, which might react with and inactivate polymerase, reacted SV40 DNA was added to nonreacted DNA that had been preincubated with polymerase. No loss of transcriptional activity was observed when compared to native DNA transcription alone (data for native FI not shown). This result is independent of CMC reaction time (Table 1). However, when nonreacted DNA was added to reacted DNA that was preincubated with polymerase, approximately 60% of the transcriptional activity was lost (Table 1). Since this inhibition did not increase significantly with increasing CMC reaction time, as the inhibition of transcription did (Fig. 1), it suggests that contaminating CMC in the reacted DNA sample may

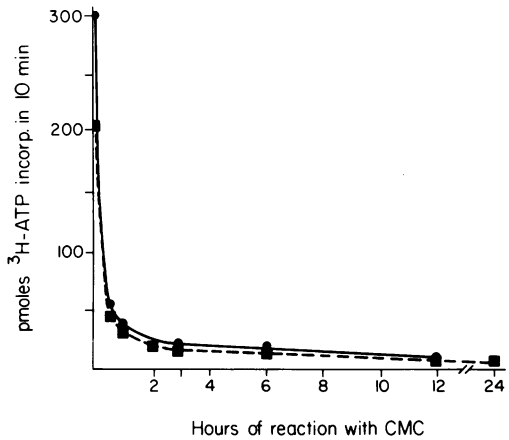

FIG. 1. *Transcription inhibition by CMC reaction. CMC reaction and transcription assay were as described in the text. Symbols: (●) SV40 FI DNA with 4 μg of rifampin per ml added with the triphosphates; (■) SV40 FI DNA with no rifampin added. All assays contained 1 μg of DNA and 25 μl of a 1:75 dilution of E. coli RNA polymerase (~6 mg/ml).*

TABLE 1. *Effects of CMC-modified SV40 DNA on the transcription of native SV40 DNA*

CMC reaction (h)	[^{3}H]ATP incorporated (pmol)		Inhibition (%)
	NR SV40I + CMC-SV40I[a]	CMC-SV40I + NR SV40I[b]	
0.5	123	49	60
2	113	36	68
3	118	39	67
6	125	35	72
12	109	56	49

[a] One microgram of NR SV40I (nonreacted SV40 DNA I) was incubated with 25 μl of polymerase, prepared by the Burgess procedure (4) (2.5 mg/ml), in assay buffer for 10 min at 37°C. One microgram of CMC-reacted SV40 DNA I (CMC-SV40I) was added, and the mixture was incubated 10 min more at 37°C. Triphosphates and 4 μg of rifampin per ml were then added, and transcription was carried out for 10 min and measured as described in the text.

[b] Same as footnote *a* except order of addition of DNA was reversed, CMC-SV40I was added to the assay mixture before polymerase addition, and NR SV40I was added after polymerase and CMC-SV40I were incubated together.

have been the cause of the inhibition. Carbodiimide reagents are known to react with proteins, and, thus, the free reagent may have been reacting with and inactivating any polymerase not bound to DNA in pre-initiation complexes.

At the 500:1 ratio of CMC to DNA used in the reaction mixture (see Materials and Methods), the maximum contamination of free CMC per microgram of DNA used in the assay would be 600 μg. The CM Bio-Gel A column step removes approximately 95% of free CMC (R. Espejo and J. Lebowitz, unpublished data); thus, 5%, or 30 μg, of free CMC would be expected to contaminate 1 μg of reacted DNA. Therefore, these two concentrations were chosen to test the effects of CMC on polymerase activity with nonreacted DNA. Table 2 shows the results of adding these CMC concentrations before and after the DNA and polymerase were incubated together. Both in the presence and in the absence of rifampin, CMC strongly inhibited the transcriptional activity of polymerase if the reagent was incubated with the polymerase for 10 min at 37°C before DNA was added and polymerase-DNA complexes were allowed to form. If, however, the DNA and polymerase were incubated for 10 min before CMC is added, no significant inhibition was observed. It appears, then, that if the CMC is able to react with the polymerase once it has formed a pre-initiation complex with DNA, it does so without affecting the ability of the complex to transcribe when triphosphates and rifampin are added.

Since the CMC reactivity with protein should be a nonspecific reaction with carboxyl groups, the order-of-addition effect should be eliminated by incubation of the contaminated reacted DNA with any protein before incubation with polymerase. The assay buffer used in the transcription assay contains approximately 125 μg of bovine serum albumin per assay mixture. Incubation of the reacted DNA for 10 min at 37°C in this buffer before polymerase was added eliminated the order-of-addition effect observed in Table 1 (Table 3). Also, Table 3 shows that, despite incubation with bovine serum albumin, reacted DNA alone showed the inhibition observed in Fig. 1. It can be concluded, therefore, that the inhibition observed in Fig. 1 is due to DNA modification and not to inhibition of polymerase activity.

Inhibition of polymerase saturation of SV40 DNA I by reaction of the DNA with CMC. The effect of CMC reaction with SV40 DNA I could occur at any, or all, of three steps

TABLE 2. *Effects of adding CMC to unbound and bound E. coli RNA polymerase on the transcription of SV40 DNA I*

Assay mixture	[^{3}H]ATP incorporated (pmol)	Inhibition (%)
Polymerase + SV40 DNA I[a]	83	
Polymerase + 600 μg of CMC + SV40 DNA I[a]	32	62
Polymerase + 30 μg of CMC + SV40 DNA I[a]	58	30
Polymerase + SV40 DNA I + Rif[a]	52	
Polymerase + 600 μg of CMC + SV40 DNA I + Rif[a]	3	94
Polymerase + 30 μg of CMC + SV40 DNA I + Rif[a]	22	59
SV40 DNA I + polymerase + Rif[b]	83	
SV40 DNA I + polymerase + 600 μg of CMC + Rif[b]	76	8
SV40 DNA I + polymerase + 30 μg of CMC + Rif[b]	80	4

[a] A 25-μl amount of a 1:75 dilution of polymerase from R. Burgess (6 mg/ml) in assay buffer was incubated with 0, 30, or 600 μg of CMC for 10 min at 37°C. One microgram of nonreacted SV40 DNA I was then added, and incubation was continued for another 10 min at 37°C. Triphosphates with or without 4 μg of rifampin (Rif) per ml were added, and transcription was allowed for 10 min and measured as described in the text.

[b] Same as footnote *a* except DNA and polymerase were incubated together for 10 min at 37°C before the addition of CMC and the subsequent incubation. Polymerase used was prepared according to the Burgess procedure (4) (2.5 mg/ml).

in transcription. (i) If the single-stranded site that reacts with the reagent is involved directly in the binding of polymerase to the DNA, binding may be blocked and transcriptional activity may be lost. (ii) If the single-stranded site is at the initiation site, the enzyme might be able to bind to the DNA after CMC modification but might not be able to begin RNA synthesis when the triphosphates are added. (iii) If the modified region is near, but distinct from, either the binding or the initiation site, premature termination could result in the production of pieces of RNA too small to be detected in the transcription assay.

TABLE 3. *Protection against CMC inactivation of E. coli RNA polymerase by preincubation of reacted DNA with bovine serum albumin*

Assay mixture[a]	[^{3}H]ATP incorporated (pmol)	Inhibition (%)
NR SV40I + BSA + polymerase	83	
0.5-h CMC-SV40I + BSA + polymerase + NR SV40I	125	0
0.5-h CMC-SV40I + BSA + polymerase (no Rif.)	21	75
NR SV40I + BSA + polymerase + Rif	52	
0.5-h CMC-SV40I + BSA + polymerase + NR SV40I + Rif	52	0
0.5-h CMC-SV40I + BSA + polymerase + Rif	5.6	90

[a] One microgram of CMC-reacted SV40 DNA I (CMC-SV40I) was incubated in an assay buffer containing 125 μg of bovine serum albumin (BSA) for 10 min at 37°C. A 25-μl amount of a 1:75 dilution of polymerase from R. Burgess (6 mg/ml) was added, and the incubation at 37°C was continued for another 10 min, followed by the addition of 1 μg of nonreacted SV40 DNA I (NR SV40I) and another 10 min, 37°C incubation. Triphosphates with or without 4 μg of rifampin (Rif) per ml were added, and transcription was allowed for 10 min and measured as described in the text.

To test which step is blocked by CMC modification, we examined the saturation of the template by polymerase as a function of CMC modification. We would anticipate two different types of saturation curves if binding were affected versus a block at initiation or premature termination. These hypothetical curves are represented in Fig. 2. For inhibition of initiation or premature termination, less RNA synthesis would occur for the fixed incubation time when there are more DNA binding sites than polymerase molecules, since some enzyme molecules would bind adjacent to a CMC defect. Consequently, we should observe a decrease in the slope of [^{3}H]ATP incorporated per microliter of polymerase added for each CMC-modified DNA. The respective CMC-modified templates should saturate at the same number of moles of enzyme. This would lead to a series of saturation curves with different slopes and equivalent break points as saturation is reached. These are represented in Fig. 2B. On the other hand, if binding were blocked by CMC, RNA polymerase could still find functional binding sites when the DNA is in excess, and the synthesis would remain identical to the native DNA. However, the equivalence point, or number of sites per DNA molecule, would be reduced, and saturation would occur at a lower number of enzyme molecules. Consequently, increased CMC modification should show the same saturation slope with different break points. This is represented in Fig. 2A.

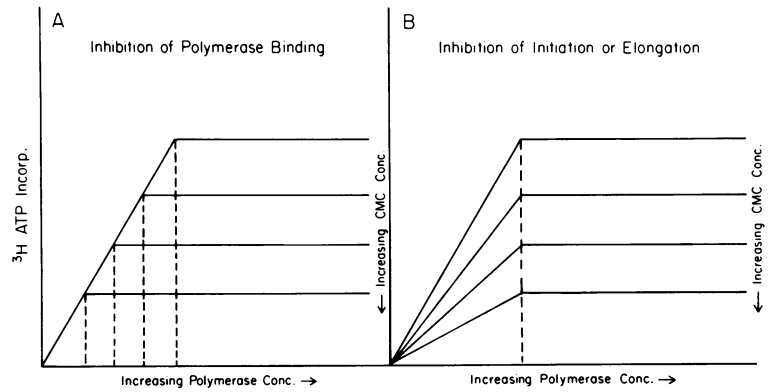

FIG. 2. *Hypothetical curves for saturation of CMC-reacted DNA by RNA polymerase. (A) Curves expected for inhibition of polymerase binding by increasing modification of the DNA by CMC reaction. (B) Curves expected for inhibition of initiation of transcription or elongation of the RNA chain by increasing modification of the DNA by CMC reaction.*

At early reaction times, the amount of polymerase needed to saturate the DNA in rifampin-resistant pre-initiation complexes decreased significantly upon increasing CMC-DNA reaction (Fig. 3). This can be seen in the change of inflection points on the curves to lower amounts of polymerase as time of reaction of DNA with CMC increases.

When the percent inhibition of saturation observed in Fig. 3 is compared to the percent inhibition of transcription on the same samples (Fig. 4), they fall on the same curve. These results indicate that the transcription inhibition observed at early reaction times may be accounted for by a loss of polymerase binding to DNA in rifampin-resistant complexes.

Saturation of 2-h CMC-reacted SV40 DNA I in the presence of 2 or 4 μg of rifampin per ml. The antibiotic rifampin may be used to select for strong promoter sites. RNA polymerase is inactivated very slowly when it is bound to a strong promoter relative to a less stable binary complex. The rate of rifampin inactivation of polymerase when it is added with the triphosphates to a polymerase-DNA complex is dependent on the ability of the enzyme to rapidly initiate RNA synthesis before the drug binds to the β subunit. The inactivation occurs by preventing initiation; consequently, tightly bound polymerase molecules will be inactivated at a lower rate than that of enzyme molecules bound less tightly to the DNA. This should allow us to use rifampin inactivation to probe the tightness of binding sites for polymerase on modified DNA. Increasing concentrations of rifampin may be used to select out weak binding sites created by CMC modification. If this is the case, we would anticipate a lower polymerase saturation point for transcription. The effects of different concentrations of rifampin on the enzyme binding to 2-h CMC-modified SV40 DNA support a differential weakening effect of CMC modification upon polymerase binding sites.

Native, nonreacted SV40 DNA shows the same saturation curve under concentrations of rifampin of 2, 4, 6, or 8 μg/ml, as shown in the accompanying paper (9). However, polymerase binding sites on the DNA become increasingly weak with CMC reaction. At 2 h of CMC reaction, the saturation curves for 2 and 4 μg of rifampin per ml appear to show this effect. This is illustrated in Fig. 5, where the 2-μg/ml rifampin (added with triphosphates) polymerase saturation curve is shifted significantly to a lower polymerase concentration than that for native, nonreacted SV40 FI DNA at 2 or 4 μg/ml. When the concentration of rifampin is increased to 4 μg/ml, the curve is shifted even lower. These

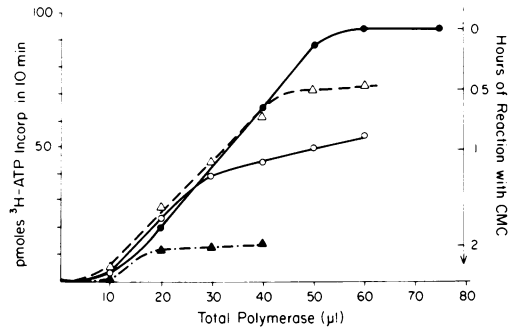

FIG. 3. *Inhibition of E. coli RNA polymerase saturation of CMC-reacted SV40 FI DNA. CMC reaction and transcription assay were as described in the text. Symbols:* (●) *No CMC reaction;* (△) *30-min CMC reaction;* (○) *1-h CMC reaction;* (▲) *2-h CMC reaction. All assays contained 1 μg of DNA, given amounts of a 1:75 dilution of E. coli RNA polymerase (~0.84 mg/ml), and 4 μg of rifampin per ml added with the triphosphates.*

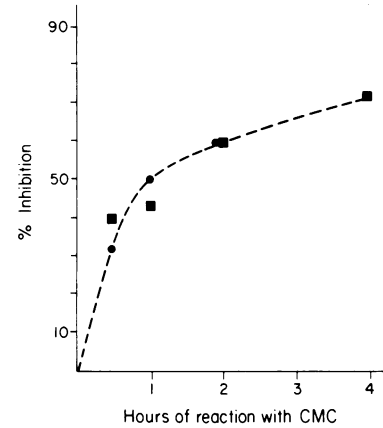

FIG. 4. *Inhibition of both saturation by E. coli RNA polymerase and transcription of SV40 FI DNA by increasing CMC reaction. CMC reaction and transcription assay were as described in the text. Symbols:* (●) *Percent inhibition of saturation;* (■) *percent inhibition of transcription. One microgram of reacted SV40 FI DNA was used in all saturation assays. All transcription assays contained 25 μl of a 1:75 dilution of E. coli RNA polymerase (~0.84 mg/ml). All saturation assays contained given amounts of the same dilution of the same batch of E. coli RNA polymerase. All assays contained 4 μg of rifampin per ml added with the triphosphates.*

results lend further support to the conclusion that, at early CMC reaction times, polymerase binding is the major transcription step inhibited by CMC modification of single-stranded regions in supercoiled DNA.

DISCUSSION

In vitro transcription of SV40 DNA I by *E. coli* RNA polymerase has been shown to be

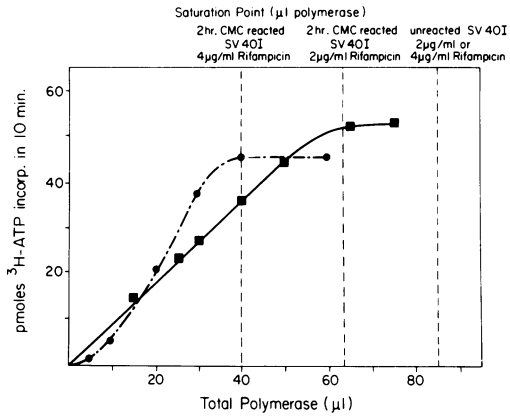

FIG. 5. *Saturation of 2-h CMC-reacted SV40 FI DNA (SV40I) in the presence of 2 or 4 μg of rifampin per ml. CMC reaction and transcription assay were as described in the text. Symbols: (■) 2-h CMC-reacted SV40I in the presence of 2 μg of rifampin per ml; (●) 2-h CMC-reacted SV40I in the presence of 4 μg of rifampin per ml. Nonreacted SV40I saturates at 85 μl of polymerase in the presence of 0 to 8 μg of rifampin per ml (see accompanying paper [9]). In all assays, rifampin was added with the triphosphates. DNA (1 μg) and given amounts of a 1:75 dilution of E. coli RNA polymerase (~6 mg/ml) were added to each assay.*

primarily asymmetric (20) and to be initiated from five ATP and about four GTP (14) sites (see accompanying paper [9]). Two of these ATP sites are in the vicinity of the initiation site for in vivo early-region 19S mRNA (at the *Hin* A–C junction). The bacterial and mammalian enzymes, however, do not appear to initiate transcription from the same sites (14). These in vitro studies have all been done with viral DNA that has had all nucleoproteins removed from it. The question as to whether, with protein intact, the initiation sites would still be distinct is unanswered. Whether or not nucleoproteins are actually present on the DNA transcribed in vivo also remains unknown. In addition, the possibility of identical binding sites but different initiation sites has not yet been explored.

For these reasons, studies of *E. coli* RNA polymerase transcription may still have a great deal of relevance to the action of mammalian RNA polymerases. For example, the early region of SV40 DNA is read five times more frequently in the chromatin of SV3T3-transformed cells, using *E. coli* polymerase. However, when SV3T3 DNA is used, the early and late SV40 genes are read with equal frequency (1). In addition, complementary RNA made by *E. coli* RNA polymerase and microinjected into mouse kidney cells produces T antigen (7). Single-stranded regions of SV40 DNA could arise from natural protein-DNA interactions as well as from supercoiling and may be involved in both in vivo as well as the in vitro transcription that we have studied here.

Inhibition of in vitro transcription by CMC reaction has also been observed with PM2 DNA (6). Also, a recent electron microscopic comparison of transcription on linear and superhelical bacteriophage λ DNA (2) has shown an increased activity of existing promoters and the production of new initiation sites at adenine-thymine-rich, easily denatured, regions on supercoiled DNA. The author (2) reports that only one of these new promoter sites, resulting from supercoiling, may be related to in vivo initiation sites on λ DNA, but this one adenine-thymine-rich initiation site may be the same as a promoter site thought to function in the repressed lysogenic state of the virus.

The advantages of unpaired or hairpin regions for polymerase binding and initiation have been described previously (6). These regions would provide specific sites that are easily recognized and that do not require the energy needed to open the DNA for access to the template bases, as in a fully paired DNA structure. By bypassing this activation step of opening an intact duplex strand, more initiation events are allowed, explaining the enhanced transcription found with supercoiled DNA.

The studies presented here have only been concerned with the mechanism of CMC reaction at early times (less than 2 h). Transcription inhibition does increase after 2 h, to a small extent, until 12 h. It has been shown that complete reaction of single-stranded regions with the reagent does not occur until after 6 h (13) and that ~2% of the DNA is reacted at this late time. From these results, it seems likely that effects on RNA elongation may begin to be observed at later reaction times. Sites less accessible to CMC reaction (fluctuating regions of single-stranded character) may become reacted after long incubations with the reagent. These regions could then act as premature termination sites for the polymerase. This possibility and further studies on the exact site and action of this inhibition of polymerase binding by CMC reaction are now in progress.

ACKNOWLEDGMENTS

This work was supported by grant CA 17077-03 from the National Cancer Institute (NCI), U. S. Public Health Service (PHS). J.L. is a recipient of PHS research career development award K04CA00141-05 from the NCI.

We thank P. Lebowitz for communication of results before publication. We also thank Marcia Flashner for helpful discussions, Candy Welsh and Thomas Tice for expert technical assistance, including preparation of RNA polymerase, and Bonnie McLay and Judy White for assistance in preparation of the manuscript.

LITERATURE CITED

1. **Astrin, S. M.** 1975. Mapping of the SV40 specific sequences transcribed *in vitro* from chromatin of SV40 transformed cells. Biochemistry **14**:2700–2711.
2. **Botchan, P.** 1976. An electron microscopic comparison of transcription on linear and superhelical DNA. J. Mol. Biol. **105**:161–176.
3. **Botchan, P., J. Wang, and H. Echols.** 1973. Effect of circularity and superhelicity on transcription from bacteriophage λ DNA. Proc. Natl. Acad. Sci. U.S.A. **70**:3077–3081.
4. **Burgess, R., and J. Jendrisak.** 1975. A procedure for the rapid, large-scale purification of *Escherichia coli* DNA-dependent RNA polymerase involving polymin P precipitation and DNA-cellulose. Biochemistry **14**:4634–4638.
5. **Chen, M., J. Lebowitz, and N. P. Salzman.** 1976. *Hin* D restriction mapping of unpaired regions in simian virus 40 superhelical DNA I: considerations regarding structure-function relationships. J. Virol. **18**:211–217.
6. **Flashner, M., M. Katopes, and J. Lebowitz.** 1977. Inhibition of transcription of supercoiled PM2 DNA by carbodiimide modification. Nucleic Acid Res. **4**:1713–1726.
7. **Graessmann, M., and A. Graessmann.** 1976. "Early" simian-virus-40-specific RNA contains information for tumor antigen formation and chromatin replication. Proc. Natl. Acad. Sci. U.S.A. **73**:366–370.
8. **Hale, P., and J. Lebowitz.** 1976. Chemical modification of supercoiled DNA: effect on the rate of transcription, p. 135–142. *In* D. Nierlich, W. Rutter, and C. F. Fox (ed.), ICN-UCLA Symposia on Molecular and Cellular Biology, vol. 5, Molecular mechanisms in the control of gene expression. Academic Press Inc., New York.
9. **Hale, P., and J. Lebowitz.** 1978. Binding and transcription of simian virus 40 DNA by DNA-dependent RNA polymerase from *Escherichia coli*. J. Virol. **25**:298–304.
10. **Hayashi, Y., and M. Hayashi.** 1971. Template activities of the φX-174 replicative allomorphic deoxyribonucleic acids. Biochemistry **10**:4212–4218.
11. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse culture cells. J. Mol. Biol. **26**:365–369.
12. **Lebowitz, J., A. Chaudhuri, A. Gonenne, and G. Kitos.** 1977. Carbodiimide modification of superhelical PM2 DNA: considerations regarding reaction at unpaired bases and the unwinding of superhelical DNA with chemical probes. Nucleic Acid Res. **4**:1695–1711.
13. **Lebowitz, J., C. G. Garon, M. C. Y. Chen, and N. P. Salzman.** 1976. Chemical modification of simian virus 40 DNA by reaction with a water-soluble carbodiimide. J. Virol. **18**:205–210.
14. **Lebowitz, P., R. Stern, P. K. Ghosh, and S. M. Weissman.** 1977. Specificity of initiation of transcription of simian virus 40 DNA I by *Escherichia coli* RNA polymerase: identification and localization of five sites for initiation with [γ-^{32}P]ATP. J. Virol. **22**:430–445.
15. **Mandel, J., and P. Chambon.** 1974. Animal DNA-dependent RNA polymerases. Studies on the reaction parameters of transcription *in vitro* of simian virus 40 DNA by mammalian RNA polymerases A1 and B. Eur. J. Biochem. **41**:367–378.
16. **Mangel, W., and H. Chamberlin.** 1974. Studies of ribonucleic acid chain initiation by *Escherichia coli* ribonucleic acid polymerase bound to T7 deoxyribonucleic acid I. An assay for the rate and extent of ribonucleic acid chain initiation. J. Biol. Chem. **249**:2995–3001.
17. **Metz, D. H., and G. L. Brown.** 1969. The investigation of nucleic acid secondary structure by means of chemical modification with a carbodiimide reagent. II. The reaction between N-cyclohexyl-N'-β-(4-methylmorpholinium)ethylcarbodiimide and transfer ribonucleic acid. Biochemistry **8**:2329–2341.
18. **Richardson, J. P.** 1974. Effects of supercoiling on transcription from bacteriophage PM2 deoxyribonucleic acid. Biochemistry **13**:3164–3169.
19. **Wang, J. C.** 1974. Interactions between twisted DNAs and enzymes: the effects of superhelical turns. J. Mol. Biol. **87**:797–815.
20. **Westphal, H.** 1970. SV40 DNA strand selection by *Escherichia coli* RNA polymerase. J. Mol. Biol. **50**:407–420.
21. **Woodworth-Gutai, M., and J. Lebowitz.** 1976. Introduction of interrupted secondary structure in supercoiled DNA as a function of superhelix density: consideration of hairpin structures in superhelical DNA. J. Virol. **18**:195–204.
22. **Zimmer, S. G., and R. L. Millette.** 1975. DNA-dependent RNA polymerase from pseudomonas Bal-31. II. Transcription of the allomorphic forms of bacteriophage PM2 DNA. Biochemistry **14**:300–306.