Effects of Supercoiling on Transcription from Bacteriophage PM2 Deoxyribonucleic Acid†

John P. Richardson

ABSTRACT: PM2 DNA, a supercoiled circular DNA, is a very effective template for RNA synthesis catalyzed by *Escherichia coli* DNA-dependent RNA polymerase. Two derivatives of this DNA without supercoils, one with single-strand breaks and the other with all the breaks resealed by DNA ligase, have only one-sixth the initial template activity of native PM2 DNA, when measured at 27° under ionic conditions that are favorable for the native DNA. Other derivatives resealed by DNA ligase under conditions giving them from 30 to 65 negative superhelical turns have nearly the same template activities as the native PM2 DNA. The lower activity of the nonsupercoiled forms is a result of a lower rate of RNA chain initiation. There is no difference in the rates of RNA chain growth on these DNAs. Changes in the structure of the DNA also alter the requirements for optimal activity. As a consequence, transcription of the nonsupercoiled derivatives relative to that of the native PM2 DNA improves when the KCl concentration is decreased, when the temperature is increased, when $MnCl_2$ is added to the reaction mixture, and when core enzyme is used in place of enzyme with σ factor. These DNAs also vary in their sensitivity to inactivation by ethidium bromide. The concentration of this DNA-binding drug needed to inhibit transcription by 50% is lower for the nonsupercoiled derivatives than it is for the native supercoiled PM2 DNA.

Many double-helical DNA molecules have been isolated as circles containing supercoils and some are known to be effective templates for RNA synthesis in the reaction catalyzed by *Escherichia coli* RNA polymerase. One example is the ϕX replicative form I DNA (Hayashi and Hayashi, 1971) and another is the DNA isolated from bacteriophage PM2 (Richardson and Parker, 1973). When ϕX replicative form I DNA is converted to a form with no supercoils by the introduction of a single-strand break (a nick), its effectiveness as a template for RNA synthesis is reduced by a factor of three. Although nicks themselves are known to affect directly the template properties of a DNA (Vogt, 1969; Hinkle *et al.*, 1972; Dausse *et al.*, 1972), the magnitude of this change with ϕX DNA suggested that a structural characteristic related to supercoiling is even more important than the presence of the nick (Hayashi and Hayashi, 1971). This interpretation is supported by the results of Botchan *et al.* (1973), who showed that circular λ DNA containing −50 to −110 superhelical turns[1] are more active templates than either linear λ DNA or the closed-circular form with no supercoils.

Since supercoiling is a characteristic of many DNA molecules including those in chromosomes of bacteria (Worcel and Burgi, 1972) and eukaryotic organisms (Pardon and Wilkins, 1972), it is important to understand the relationship between this structural feature and the template functions of DNAs. This paper reports experiments concerning this relationship in PM2 DNA, which is a relatively small (mol wt 6.3×10^6) DNA that contains about −51 superhelical turns in its native form (Gray *et al.*, 1971). It is shown that derivatives of PM2 DNA with no supercoils and either with or without nicks are much less active templates for RNA synthesis catalyzed by *E. coli* RNA polymerase than native PM2 DNA or derivatives containing −30 to −65 superhelical turns. Some of the possible reasons for the low activity of the nonsupercoiled forms are examined by comparing number of chains initiated, the rates of RNA chain growth, the ionic conditions for optimal activity, the effects of σ factor, and the sensitivity to inactivation by ethidium bromide with the different forms of DNA.

Materials and Methods

RNA polymerase was isolated from *E. coli B* as described previously (Richardson, 1966a, 1973), and its σ factor was separated from core enzyme by chromatography on phosphocellulose (Burgess and Travers, 1971; Berg *et al.*, 1971). DNA ligase (fraction VII) was isolated from T4-infected cells by the method of Weiss *et al.* (1968).

Native PM2 DNA (form I) was isolated by phenol extraction of purified PM2 virus as described (Richardson, 1973). The preparations used had greater than 97% closed circular DNA molecules. A derivative containing single-strand nicks (form II) was prepared by incubating 270 μg of PM2 DNA with 7.5 ng of pancreatic deoxyribonuclease (Worthington, electrophoretically pure, diluted in a solution containing 0.05 M potassium phosphate buffer (pH 5.9), 5 mM $MgCl_2$, and 0.5 mg/ml of bovine serum albumin) in 1 ml of a solution containing 5 mM $MgCl_2$, 0.02 M Tris-HCl buffer (pH 7.2), and 0.05 M NaCl. After 15 min at 37°, the reaction was stopped by adding 5 μl of 10% sodium dodecyl sulfate, and this mixture was layered directly on a 15-ml sucrose gradient (20–5%, w/v) in 0.1 M NaCl, 0.02 M Tris-HCl buffer (pH 8.0), and 1 mM Na_3EDTA. After centrifugation for 15 hr at 27,000 rpm and 4° in a Beckman SW41 Ti Rotor, fractions were collected and those containing form II (20 S) were pooled. To concentrate the DNA, NaCl was added to 0.5 M followed by two volumes of absolute ethanol and after 3 hr at −20° the nearly invisible precipitate was collected by centrifugation in Corex tubes for 30 min at 18,000 rpm in the Sorvall SS-34 rotor. The pellets were dried for 1 hr in a vacuum desiccator over KOH pellets before resuspension in 0.5 ml of D buffer (0.05 M KCl–0.01 M Tris-HCl buffer (pH 8.0)–0.5 mM Na_3EDTA). The remaining ethanol and sucrose were removed by dialysis for 15 hr against D buffer. This treatment converted approximately 70%

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[1] The superhelical turns are in the negative sense because they are opposite to the right-handed DNA helix.

of the DNA to form II, of which 62% was recovered by this procedure. Assuming a random distribution for the nicks in the preparation of form II, 51, 30, 12, and 7% of the molecules had 1, 2, 3, and more than 3 nicks, respectively.

Closure of DNA by Ligase. Closed circular PM2 DNA with no supercoils (form I^0) was prepared by the action of DNA ligase on PM2 form II DNA under the following conditions. A 1-ml reaction mixture contained 120 μg of PM2 form II DNA and 0.6 unit of ligase (see Weiss *et al.*, 1968) in 75 mM Tris-HCl buffer (pH 7.6), 6.5 mM $MgCl_2$, 0.1 mM ATP, and 5.5 mM dithiothreitol. After incubation for 30 min at 37°, 32 μl of 0.4 mM Na_3EDTA was added and the mixture was heated for 5 min at 60°. Form I^0 DNA was separated from unreacted form II DNA by banding in CsCl gradients containing ethidium bromide (Radloff *et al.*, 1967). After isolation of the form I^0 band, ethidium bromide was removed by extraction with isobutyl alcohol followed by dialysis for 48 hr against D buffer containing 1 M KCl. The DNA was equilibrated with D-buffer dialysis before use. The overall yield was about 50%. Some preparations that had less than 10% unsealed DNA after ligase treatment were used without the ethidium bromide–CsCl isolation step. Identical results were obtained with PM2 I^0 DNA prepared either way.

Derivatives with varying numbers of superhelical turns were prepared in separate 0.75-ml reaction mixtures each containing 50 μg of DNA and 0.4 unit of ligase and either 3.1, 6.4, 9.8, 13.5, 17.5, or 24 nmol of ethidium bromide. After incubation in the dark for 30 min at 37°, the closed PM2 DNA derivatives were isolated by the banding procedure described for the form I^0 DNA.

The number of superhelical turns in a PM2 DNA derivative was determined by density differences in CsCl-ethidium bromide density gradients. All measurements of superhelix densities (the number of superhelical turns per ten base pairs) were made relative to native (viral) PM2 DNA using the relationship between density separation and superhelix density determined by Gray *et al.* (1971). It was assumed that the superhelix density of the native PM2 DNA is −0.053 at 20° in 2.83 M CsCl (Gray *et al.*, 1971). Since the molecular weight of PM2 DNA is 6.3×10^6 daltons, this corresponds to −51 superhelical turns. The number of superhelical turns under the condition used for RNA synthesis has not been determined, but from the known effects of salt and temperature on the average rotation per base pair of the DNA helix (Wang, 1969) there are probably fewer turns in native PM2 DNA under the condition used for RNA synthesis than in 2.83 M CsCl. However, the DNA that was closed with ligase under conditions used for RNA synthesis presumably has no superhelical turns under those conditions. It was also found to have no superhelical turns by the density difference method of Gray *et al.* (1971). Thus, even if the number of superhelical turns in native PM2 DNA is not exactly −51, it is substantial compared with the number in form I^0 DNA.

T4 DNA and T7 DNA were prepared by the method of Thomas and Abelson (1966) and poly(dA-dT) was purchased from Miles Laboratories.

RNA Synthesis Measurements. The standard RNA synthesis reaction mixtures contained, in a final volume of 0.1 ml, 0.1 M KCl, 0.01 M $MgCl_2$, 0.04 M Tris-HCl buffer (pH 8.0), 0.4 mM each ATP, GTP, CTP, and [^{3}H]UTP (10 Ci/mol), 10^{-4} M dithiothreitol, 10^{-4} M EDTA, 1 μg of DNA, and 1 μg of RNA polymerase. All components except the nucleoside triphosphates were mixed together in a final volume of 80 μl and preincubated for 20 min at 27° before adding the nucleoside triphosphates in a final volume of 20 μl. After 5 min more at 27° (or 60 min, in some cases) RNA synthesis was terminated by the rapid addition at 0° of 0.5 ml of 0.1 M $Na_4P_2O_7$ and 0.5 ml of 10% trichloroacetic acid. Precipitates were collected and washed on membrane filters (0.45-μm pore size) and radioactivity was determined by scintillation counting. The efficiency of counting for tritium was 36%. Total nucleotide incorporation was calculated from the amount of [^{3}H]UMP incorporated assuming the mole ratio for UMP in PM2 RNA is identical with the mole ratio of 0.285 for dTMP in PM2 DNA (Espejo *et al.*, 1969).

In experiments using γ-[^{32}P]ATP (prepared by the method of Glynn and Chappell, 1964), background incorporation was reduced further by soaking washed filters for 1 hr at 0° in 20 ml of a solution containing 1 M KCl, 0.01 M $Na_4P_2O_7$, and 5% trichloroacetic acid before final rinsing and drying (Travers and Burgess, 1969).

RNA chain growth measurements were made as described previously (Richardson, 1973). This method was altered slightly from the earlier technique (Richardson, 1970) to ensure complete denaturation of the RNA by formaldehyde, otherwise not all the RNA was released from the DNA.

TABLE I: Template Activities of PM2 DNA and Its Derivatives.

PM2 DNA Type	Form	No. of Superhelical Turns[a]	[^{3}H]UMP Incorpd (pmol) At 5 min	At 60 min
Native	I	−51	126	401
Nicked	II	0	22	199
Resealed	I^0	0	18	182
	I^1	−12	51	334
	I^2	−26	117	416
	I^3	−44	117	409
	I^4	−55	124	346
	I^5	−64	119	298
	I^6	−75	100	261

[a] These are based on the value of −51 for PM2 DNA at 20° in 2.83 M CsCl, and are thus presumably appropriate for that solvent and temperature.

Results

Supercoiling and Template Activity. The relative template activity of a DNA in the RNA polymerase catalyzed reaction is determined from the amount of RNA synthesized under standard assay conditions with DNA limiting. Table I shows the amounts of RNA synthesized after 5- and 60-min incubations using several forms of PM2 DNA containing different numbers of superhelical turns. The least active of all the templates is the closed circular PM2 DNA with no supercoils (form I^0). It is even less active than the nicked derivative (form II). After 5 min at 27°, nearly ten times as much RNA is synthesized from the native PM2 DNA (form I) than is synthesized from form I^0 DNA. However, with a longer incubation the discrepency is not so great: after 60 min the ratio of RNA synthesized from form I DNA to that synthesized from form I^0 DNA is 2.2.

Full activity is restored when the nicked derivative (form II) is resealed with −30 to −65 superhelical turns; derivatives I^2, I^3, I^4, and I^5 have almost the same template activities as form I DNA. This result indicates that the structural constraints of PM2 DNA that give rise to the supercoils are important for template activity. However, the requirements for this structur-

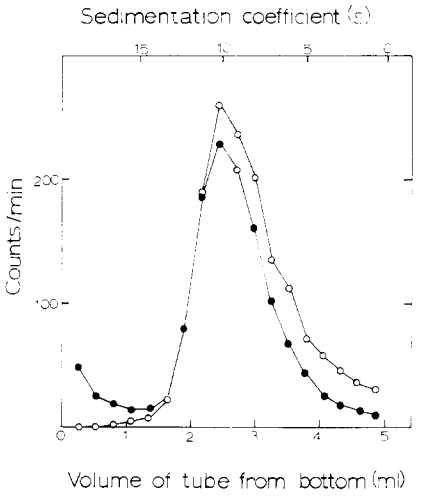

FIGURE 1: Zone sedimentation profiles of RNA synthesized from PM2 form I and form I^0 DNA templates on gradients containing formaldehyde. RNA was synthesized in two separate standard reaction mixtures. One contained 1 μg of PM2 form I DNA and [^{14}C]UTP (20 Ci/mol), and the other contained 1 μg of PM2 form I^0 DNA and [^{3}H]UTP (200 Ci/mol). After 3 min of incubation (at 27°) with nucleoside triphosphates, 8 μl of a solution containing 4% sodium dodecyl sulfate in 0.4 M Na_3EDTA was added to each. After 5 min at 27°, 20 μl of the form I reaction mixture was combined with 45 μl of the form I^0 reaction mixture, 10 μl of a solution containing 40 μg of *E. coli* total RNA, and 15 μl of 11 M HCHO in 0.1 M sodium phosphate buffer (pH 7.7). After incubation for 10 min at 70°, 60 μl of 0.23 M sodium phosphate buffer (pH 7.7) was added and the mixture was centrifuged on a 5-ml sucrose gradient containing 1.1 M HCHO in 0.1 M sodium phosphate buffer (pH 7.7) as described previously: (●) [^{14}C]RNA (from PM2 I DNA); (○) [^{3}H]RNA (from PM2 I^0 DNA).

al constraint are not very critical because the range of supercoiling in the active derivatives is wide.

Initiation and Growth of RNA Chains. The initial rate of RNA synthesis depends on the number of enzyme molecules that can act and the rate of RNA chain growth. Within experimental error, the rates of growth of RNA from different PM2 DNA templates, estimated from the sedimentation properties of the RNA as a function of time (Bremer and Konrad, 1964; Richardson, 1970, 1973), are the same; in standard reaction mixtures with nucleoside triphosphates at 0.4 mM each, PM2 RNA chains grow at rates of 8.2, 8.5, and 9.3 (±1.0) nucleotides per second from form I, form II, and form I^0 DNA, respectively, at 27°. The identity of RNA growth rates from form I and form I^0 DNA templates is shown in Figure 1 by the co-sedimentation of ^{3}H-labeled 3-min RNA synthesized from form I^0 PM2 DNA with ^{14}C-labeled 3-min RNA synthesized from form I PM2 DNA. This experiment confirms the results

TABLE II: Initiation of PM2 RNA Chains with ATP.

PM2 DNA	Time (min)	[^{3}H]UMP Incorpd (nmol)	γ-[^{32}P]ATP Incorpd (pmol)
Form I (native)	20	0.38	0.34
	40	0.46	0.46
Form II	20	0.10	0.11
	40	0.16	0.18
Form I^0	20	0.11	0.10
	40	0.19	0.12
Form I^6	20	0.36	0.35
	40	0.51	0.46

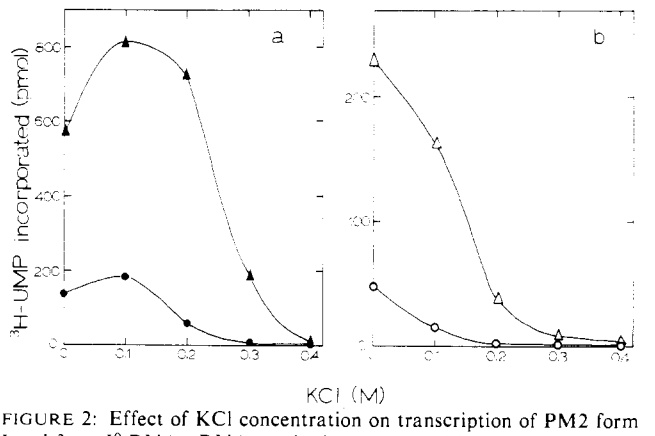

FIGURE 2: Effect of KCl concentration on transcription of PM2 form I and form I^0 DNAs. RNA synthesis was assayed in standard reaction mixtures with different concentrations of KCl: (a) form I DNA; (●) 5-min incubation; (▲) 60-min incubation; (b) form I^0 DNA; (○) 5-min incubation; (△) 60-min incubation.

of similar experiments with λ DNA reported by Botchan *et al.* (1973).

Since the rates of polymerization are the same on the three forms of PM2 DNA, the decrease in the rates of synthesis from the nonsupercoiled forms must arise from a reduction in the number of RNA chains initiated. Direct measurements of the number of chains initiated from different PM2 DNA templates support this conclusion. Even after 20 and 40 min, fewer chains are initiated with ATP (normally, two-thirds of the PM2 RNA chains are initiated with ATP (Richardson, 1973)) from the PM2 forms II and I° DNA templates than from the native PM2 DNA or the resealed form I^6 DNA (Table II). Within the uncertainty of these measurements (±0.05 pmol for ATP incorporation), the reduction in chains initiated with ATP corresponds to the decrease in the amounts of RNA synthesized.

Differences in the Reaction Requirements. The conditions chosen for the comparisons made in Tables I and II are those favorable for the use of native (form I) PM2 DNA as a template. Figure 2a shows that this template is most active in 0.1 M KCl. In contrast, form I^0 DNA is most active when no KCl is added (Figure 2b). The effect of KCl on transcription of form II DNA is similar to that for form I^0 DNA (data not shown).

In 0.1 M KCl, there is no significant difference in the optimum $MgCl_2$ concentrations for these three PM2 DNA tem-

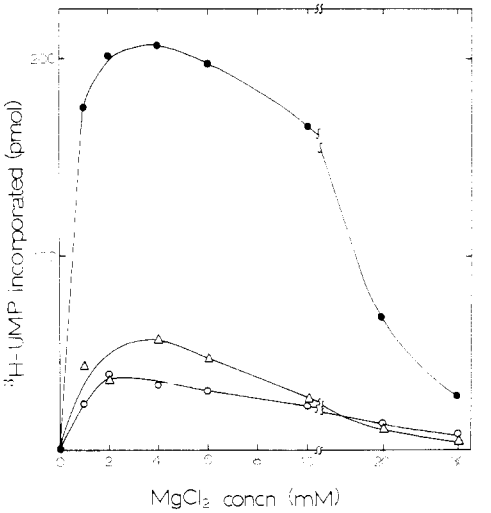

FIGURE 3: Effect of $MgCl_2$ on transcription of different forms of PM2 DNA. RNA synthesis was assayed in standard reaction mixtures with 5-min incubations: (●) form I DNA; (△) form II DNA; (○) form I^0 DNA.

TABLE III: Effects of $MnCl_2$ on Transcription of PM2 DNAs.[a]

Divalent Cations	[^{3}H]UMP Incorpd (pmol)		
	Form I DNA	Form II DNA	Form I^0 DNA
4 mM $MgCl_2$	168	51	30
4 mM $MgCl_2$ + 1 mM $MnCl_2$	133	73	42

[a] Standard RNA synthesis conditions with 5-min incubations were used with divalent cations as indicated in the table.

plates tested (Figure 3). However, these DNAs do differ in their responses to $MnCl_2$. Addition of 1 mM $MnCl_2$ stimulates transcription of the nonsupercoiled DNAs, but inhibits transcription of form I PM2 DNA (Table III). This result conforms with a general observation (unpublished results) that Mn^{2+} inhibits transcription of active templates with *E. coli* RNA polymerase whereas it stimulates transcription of poorer templates, such as denatured DNA.

The temperature of 27° used for the experiments of Table I is optimal for growth of PM2 virus. However, all the PM2 DNA derivatives gave higher rates of RNA synthesis *in vitro* when the temperature was increased to 37°. The temperature activation for native PM2 DNA is almost identical with that for native T7 DNA (Figure 4). However, the degree of activation is greater for both form I^0 and form II DNAs. The ratios of activity at 37° relative to that at 20° are 4.5, 15, and 18 for forms I, II, and I^0 of PM2 DNA, respectively.

Effect of the σ Factor. Since the σ factor is required for initiation of transcription from certain DNA sites but not from others, the effect of this protein on transcription of different forms of PM2 DNA has been tested to determine some of the characteristics of their initiation sites. The results of Table IV show that core RNA polymerase, which has good activity with poly(dA-dT), has very low activity with the three forms of PM2 DNA tested, although in none of the cases is the activity as low as that found with T4 DNA. Yet even with core, the nonsupercoiled forms are less active than form I DNA. Thus,

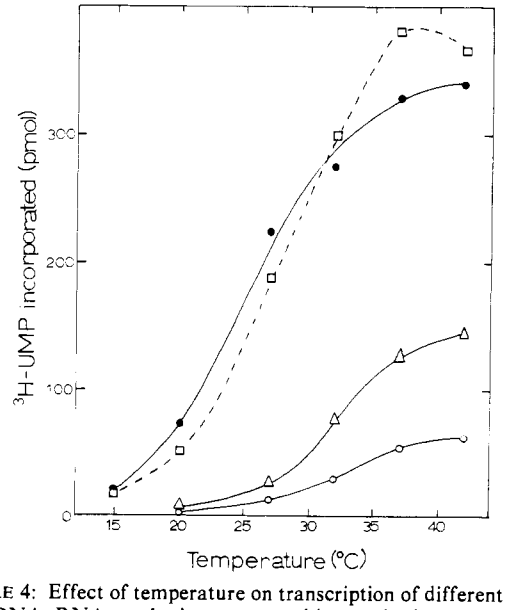

FIGURE 4: Effect of temperature on transcription of different forms of PM2 DNA. RNA synthesis was assayed in standard reaction mixtures using the different temperatures for both preincubation (20 min) and for nucleotide incorporation (5 min): (●) PM2 form I DNA; (△) PM2 form II DNA; (○) PM2 form I^0 DNA; (□) T7 DNA.

TABLE IV: Effect of σ Factor on Transcription of Different Forms of PM2 DNA.[a]

DNA	pmol of [^{3}H]UMP Incorpd		
	Core enzyme	Standard enzyme	Standard enzyme + 1 μg of σ
PM2 I	22	126	214
PM2 II	11	18	42
PM2 I^0	13	31	52
T4	5	81	116
Poly(dA-dT)	232	273	325

[a] RNA synthesis was measured in a standard reaction mixture containing 1 μg of RNA polymerase (either core or standard enzyme) incubated for 5 min. "Standard enzyme" was unfractionated pure RNA polymerase; the preparation used contained 0.45 equiv of σ. The core RNA polymerase contained <0.8% σ by weight (<0.04 equiv), and σ had <10% core. A mixture of equal weight of σ and standard enzyme has about 5 σ equivalents per core. In the reaction mixtures containing poly(dA-dT), GTP and CTP were omitted.

unlike the addition of nicks to T4 DNA or T7 DNA, loss of supercoiling does not activate PM2 DNA for transcription with core enzyme. However, the differences in activity with core are not as great as with enzyme containing σ, and stimulation of transcription of the nonsupercoiled templates by σ is less than that found with form I DNA. Hence, some of the residual activity from the poorer PM2 templates may be from σ-independent sites.

Effects of Ethidium Bromide on Transcription of PM2 DNAs. Another important difference in the template properties of native PM2 DNA and its derivatives is found in their responses to inhibition by ethidium bromide, a drug that selectively affects initiation at certain DNA sites (Richardson and Parker, 1973). With a DNA concentration of 7.5 nmol/ml, transcription of native PM2 DNA is inhibited by 50% in 2.0 μM ethidium bromide (Figure 5). Both of the derivatives with

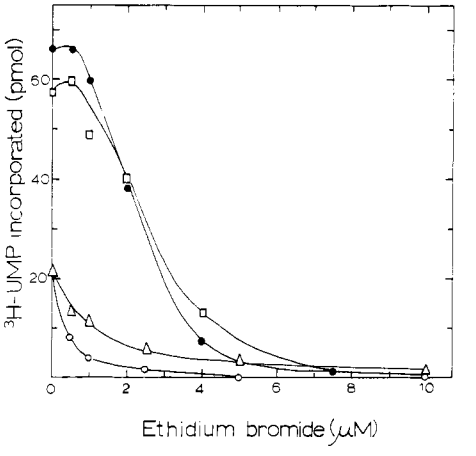

FIGURE 5: Effect of ethidium bromide on transcription of different forms of PM2 DNA. The standard reaction conditions were altered in the following ways to be consistent with previous studies on the effects of ethidium bromide on DNA transcription (Richardson and Parker, 1973). The amount of DNA used in each assay was 0.25 μg, the concentration of the four nucleoside triphosphates was 0.2 mM each, and they were added, in a volume of 10 μl, to 90 μl of the enzyme-DNA mixtures preincubated for 5 min at 37°; incubation was for 5 min at 37°: (●) form I DNA; (△) form II DNA; (○) form I^0 DNA; (□) form I^6 DNA.

no supercoils are found to be even more sensitive to inactivation with this DNA-binding drug, even though they bind less of it at low concentrations than form I DNA (Bauer and Vinograd, 1971).

Some differences between the closed form with no supercoils (form I^0) and the form with single-strand breaks are also apparent in this experiment. Only 0.4 μM ethidium bromide is required to inhibit the transcription of form I^0 by 50%, and its template activity is completely abolished in 5 μM ethidium bromide. With form II DNA, 1.0 μM ethidium bromide is required to inhibit transcription by 50% and as much as 7% residual activity is found in 10 μM ethidium bromide, which is enough drug to inhibit transcription of form I DNA completely. Apparently, not all the initiation sites on form II DNA are as sensitive to the presence of ethidium bromide as the sites on form I^0 DNA. Perhaps the less sensitive sites are at nicks in this DNA.

Figure 5 also shows that the transcription of a resealed derivative containing about 1.5 times as many superhelical turns as native PM2 DNA is inhibited by ethidium bromide with a similar dose–response curve. Since it is the interaction between ethidium bromide and the initiation sites that is responsible for the major inhibitory effect of this drug (Richardson, 1973), the result of this experiment suggests that this interaction changes very little when the number of superhelical turns in PM2 DNA is increased from −51 to −75, even though this increase does affect the overall binding of the drug. This result also shows that the inhibition of transcription of a closed circular DNA is not directly related to the number of ethidium bromide molecules needed to remove all of the superhelical turns.

Discussion

The correlation between supercoiling and template activity of a circular double-helical DNA was first recognized by Hayashi and Hayashi (1971) as a result of their experiments with ϕX RFI DNA and its nicked derivative, ϕX RFII DNA. In this paper, a similar correlation is demonstrated for PM2 DNA. A nick introduced into a closed circular DNA with pancreatic DNase allows one strand of this DNA to wind with respect to the other, thus relaxing the constraint responsible for supercoiling. With ϕXRF and PM2 DNAs, the closed supercoiled forms are much more active than the nicked form. Although nicks themselves are known to directly inhibit transcription from some DNA template, the major effect of the few nicks that reduce the activity of PM2 DNA so dramatically appears to be related to the loss of supercoiling. This is shown by the finding that the lost activity is not recovered when the nicks are resealed unless the resealing is done under conditions that reintroduce −30 to −65 superhelical turns into the resulting closed DNA derivatives. Since full activity is restored in these cases, the nicks do not irreparably inactivate the DNA.

The nicks themselves also affect the DNA in some less dramatic ways that are apparent from the differences in template characteristics of form II and form I^0 PM2 DNAs. Form II DNA is more active than form I^0 DNA and it is also less sensitive to further inactivation by ethidium bromide. These differences probably arise from use of the randomly placed nicks as sites for initiation of RNA synthesis. Normally such sites are very inefficient (Hinkle *et al.*, 1972), but on templates as poor as the nonsupercoiled PM2 DNA they are efficient enough to have a significant effect on transcription.

It is not clear yet whether a similar structural feature is also important for the template activity of circular DNAs *in vivo*. With PM2 virus, both closed and nicked circular forms of PM2 DNA are found during all stages of infection (Espejo *et al.*, 1971), but it is not known which forms are responsible for RNA synthesis or whether the closed form is actually supercoiled in the cell. With S13-infected *E. coli*, it is known that the rate of S13 RNA synthesis is highest when most of the S13 DNA is in the closed circular RFI form and decreases when the RFI is converted to the nicked RFII (Puga and Tessman, 1973). This result suggests that there may be some special characteristic of S13 RFI DNA that makes it more active than RFII DNA *in vivo*. However, so little is known about the structure of S13 RFI DNA in the cell that it is not clear yet whether the special characteristic is related to the fact that this DNA is supercoiled when it is isolated.

The results of the RNA chain growth and chain initiation experiments indicate the template activity is lower on the nonsupercoiled derivatives because fewer RNA polymerase molecules function on these DNAs. The changes in structure related to the loss of supercoiling thus decrease the overall efficiency of initiation. There are two distinctive differences in structure between native PM2 DNA and its nonsupercoiled derivative that could account for more rapid initiation of RNA synthesis on the former. One is that native PM2 DNA has regions that react with reagents specific for single-stranded DNA while the nonsupercoiled PM2 DNA does not have such regions (Dean and Lebowitz, 1971; Beerman and Lebowitz, 1973). Since RNA polymerase is known to have a high affinity for single-stranded DNA (Richardson, 1966b), these regions may be the sites normally recognized by RNA polymerase on PM2 DNA. Another difference is that it is energetically more favorable to start to unwind the DNA helix of a circular DNA with negative supercoils than a DNA with no supercoils (Bauer and Vinograd, 1968; Davidson, 1972). Since there is evidence that RNA polymerase unwinds DNA as a step in RNA initiation (Walter *et al.*, 1967; Saucier and Wang, 1972), this step could be easier and more rapid on native PM2 DNA than on a nonsupercoiled derivative. Mapping the locations of the single-strand regions and the sites of initiation on PM2 DNA might be one way to distinguish between these possibilities. Another approach is to compare the effects of temperature and ionic conditions on the structure of DNA with their effects on the initiation of DNA chains. The experiments presented in this paper show that the transcription of native PM2 DNA responds differently to changes in temperature and ionic conditions than do the forms without supercoils, presumably because of the differences in the initiation sites used.

The overall efficiency of transcription of a DNA depends on the number of active initiation sites and the specific efficiency of each site. Any changes in the number or relative activity of different sites will alter the selectivity of transcription of the DNA. With λ DNA there is evidence that transcription is more selective from the circular form with no supercoils than from derivatives prepared to contain −110 or −160 superhelical turns (Botchan *et al.*, 1973). This result suggests that the increased template activity of supercoiled λ DNA *in vitro* may be an artifact. In order to determine whether this is a general conclusion, it is necessary to study the effects of supercoiling on the selectivity of transcription from several DNAs. Since PM2 DNA is relatively small and is naturally supercoiled in its native, viral form, it should be a good example to study.

The fact that several DNA templates have been found to be most active in their supercoiled form *in vitro* suggests that this is a characteristic of all DNAs that are normally circular, including the chromosomal DNA of *E. coli*. If this supposition is correct, it provides an explanation for the relatively poor template activity of *E. coli* DNA *in vitro* (Travers *et al.*, 1970), because the isolated DNA normally used is highly fragmented

and not supercoiled. However, supercoiling is not a necessary condition for efficient transcription of all DNAs, and some of the most active templates for *E. coli* RNA polymerase are the linear DNAs isolated from bacteriophages T4, T5, and T7. Apparently there are two distinct classes of efficient initiation sites. The sites in one class require a structural constraint in the DNA related to supercoiling in order to function well, whereas the sites in the other class do not need this constraint. It will be of interest to learn how the sites in these two classes differ in their primary structures.

Acknowledgment

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Studies on Competitive Binding of Lectins to Human Erythrocytes†

Tsutomu Kawaguchi, Isamu Matsumoto, and Toshiaki Osawa*

ABSTRACT: The binding to human group O erythrocytes of four ^{131}I-labeled lectins [*Maackia amurensis* hemagglutinins (MAM and MAH), *Ricinus communis* hemagglutinin (RCH), and *Bauhinia purpurea* hemagglutinin (BPH)] was studied in detail. Competitive binding studies permitted an analysis of the relationship between the cell receptor sites of the lectins. We conclude that MAM and RCH, or MAH and BPH, share a common oligosaccharide chain on the erythrocyte surface and bind to the same or overlapping portions of the oligosaccharide chain.

The lectins have been extensively investigated because of several peculiar biological activities. These include blood group specific hemagglutinating activity, tumor cell specific agglutinating activity, and mitogenic activity against peripheral lymphocytes (Boyd, 1963; Lis and Sharon, 1973). These activities are assumed to stem from the initial binding of the lectins to receptor sites of carbohydrate nature on the cell surface. In previous studies (Fukuda and Osawa, 1973), we have tested the antigenic receptor activity of a major glycoprotein of human group O erythrocyte membrane and suggested that the antigenic receptor sites for some lectins mostly reside in *N*-glycosidically linked oligosaccharide chains of the glycoprotein similar to the one isolated by Kornfeld and Kornfeld (1970), whereas those for the other group of lectins possibly reside in O-glycosidically linked oligosaccharide chains of the glycoprotein such as the one reported by Thomas and Winzler (1969).

† From the Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan. *Received February 22, 1974.* This investigation was supported by a research grant from the Ministry of Education of Japan.