Hin D Restriction Mapping of Unpaired Regions in Simian Virus 40 Superhelical DNA I: Considerations Regarding Structure-Function Relationships

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Received for publication 29 September 1975

Superhelical simian virus 40 (SV40) DNA I was reacted with *N*-cyclohexyl-*N'*- β -(4-methylmorpholinium)ethylcarbodiimide (CMC), and the location of CMC sites was mapped using the *Hin* D restriction endonuclease. The use of ¹⁴C-labeled CMC allows a quantitative analysis of the binding to the respective *Hin* D restriction endonuclease fragments. The percentage of reactivity was 6.54% for fragment A, 3.87% for fragment B, and 2.74% for fragment G. No CMC radioactivity was detected in other fragments. This reactivity is in agreement with the evaluation of binding by buoyant density measurements. The above fragments also contain the sites susceptible to *S*₁ endonuclease action. This adds further support to the view that superhelical DNA can contain regions of localized interrupted secondary structure which may be capable of forming intrastrand hairpin structures if sequence relationships are favorable. The possible structure-function relationships for this model are discussed with the emphasis on transcription.

In the previous paper (15) we showed that superhelical simian virus 40 (SV40) DNA I can be modified with *N*-cyclohexyl-*N'*- β -(4-methylmorpholinium)ethylcarbodiimide (CMC). CMC reacts preferentially with the imino sites of unpaired thymine and guanine residues to form a stable covalent product in the neutral pH range (19, 20). Consequently, one can map the locations of CMC on SV40 DNA I. This involves locating those *Hin* D restriction endonuclease fragments containing CMC. A comparison can then be made between the CMC reactive sites and those sites that are cleaved by single-strand-specific *S*₁ endonuclease action.

If a correspondence exists between the regions of reactivity of CMC, single-strand-specific endonucleases (3, 15), and gene 32 protein (21, 22), this would add further support to the view that superhelical DNA can contain regions of localized interrupted secondary structure which may be capable of forming intrastrand hairpin structures. An examination of possible structure-function relationships for the model proposed are discussed in the context of recent studies, particularly transcription data.

MATERIALS AND METHODS

Cell culture, SV40, and DNA preparation. Full details for the above procedures have been described

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previously (15).

Enzymes. The endonuclease *R*-*Hin* D was purified by the method of Smith and Wilcox (25) and was stored at -20°C in 0.2 M KCl-0.01 M Tris (pH 7.5)-50% glycerol at 6 U/ml.

Chemicals. Cold CMC was ordered from the Aldrich Chemical Co., Milwaukee, Wis.; ¹⁴C-labeled CMC was prepared by the New England Nuclear Corp. (Boston, Mass.) custom synthesis laboratory using 1-cyclohexyl-3-[2-morpholino-(4-ethyl)] carbodiimide that was prepared by us following previous procedures (19). This intermediate carbodiimide was converted to CMC by reaction with ¹⁴C-labeled CH₃I in the New England Nuclear Corp. laboratory.

Electrophoresis. All of the electrophoretic runs were carried out in an EC vertical-gel electrophoresis apparatus (model E-C 470). Samples (0.05 ml) were applied to slab gels (17 by 12 by 0.30 cm) consisting of 5% polyacrylamide and 0.5% agarose. Electrophoresis was carried out for 16 h at 20°C at 50 V in a buffer consisting of 40 mM Tris-hydrochloride, 20 mM sodium acetate, and 1 mM EDTA adjusted to pH 7.2 with acetic acid. To quantitate radiolabeled bands, the gels were frozen and sliced into 1-mm segments. Each segment was dissolved in 0.2 ml of 30% H₂O₂ and counted with Triton X-100 toluene scintillation fluid.

Analysis of the binding of ¹⁴C-labeled CMC to SV40 DNA. A defined amount of SV40 DNA I was placed in the gel slab after *Hin* D digestion. Since the weight fraction of each fragment is known, the calculation of binding was performed, e.g., for fragment A, as follows. The A fragment is 22.5% of the SV40 genome and consequently the gel contained

2.25 μg of A. Since only T and G are capable of reacting we have 1.125 μg or 0.00338 μmol of possible bases as potential covalent binding sites. The amount of ^{14}C -labeled CMC bound after background subtraction was 292 counts/min, which was converted into disintegrations per minute per micromole using the specific activity for CMC, 2 mCi/mmol, and the determined counting efficiency of 30%. In the case of the A fragment, 0.000212 μmol of CMC was bound, giving a reactivity of 6.54%. Similar calculations were performed for the B and G fragments.

RESULTS

Electrophoretic analysis of a *Hin* D digest of SV40 DNA I modified with unlabeled CMC. The first experiment utilized ^3H -labeled SV40 DNA I treated with unlabeled CMC (15). The *Hin* D digestion was carried out in the presence of untreated ^{14}C -labeled SV40 DNA after removal of excess CMC. Figure 1 shows that the

electrophoretic mobilities of the A and B fragments are clearly slowed by one fraction. There is an indication that the G fragment is also displaced by the CMC reaction. To confirm these assignments, it was necessary to obtain labeled CMC. This was provided to us as ^{14}C -labeled CMC by custom synthesis from the New England Nuclear Corp. (see Materials and Methods).

Electrophoretic analysis of the *Hin* D digest of ^3H -labeled SV40 DNA I modified with ^{14}C -labeled CMC. The reaction of ^{14}C -labeled CMC was carried out under conditions similar to those that were used for the reaction between cold reagent and ^3H -labeled SV40 DNA. After the excess reagent was removed, the ^3H -labeled DNA, with covalently bound ^{14}C -labeled CMC, was digested in the presence of ^{32}P -labeled SV40 DNA. The results of this triple-label experiment are shown in Fig. 2. It can readily be

FIG. 1. ^3H -labeled SV40 DNA I (specific activity, 5300 counts/min per μg) was reacted with an 800-fold excess of cold carbodiimide (moles per mole of nucleotide) in a buffer containing 0.01 M $\text{Na}_2\text{B}_4\text{O}_7$, 0.1 M Na_2SO_4 , 0.25 M NaCl, pH 8.0. The excess carbodiimide was removed by dialysis against the same buffer. The carbodiimide-reacted ^3H -labeled ($\square$) SV40 DNA I was dialyzed against 6.6 mM Tris, 50 mM NaCl (pH 7.4) before being cleaved with endonuclease *Hin* D. ^{14}C -labeled (+) SV40 DNA I marker digest (specific activity 16,000 counts/min per μg) was cleaved simultaneously in the same *Hin* D before electrophoresis in 5% acrylamide-0.5% agarose in an EC vertical-gel electrophoresis apparatus for 16 h. Gels were then sliced into 1-mm segments and dissolved in 0.3 ml 30% H_2O_2 overnight at 65 C. Ten milliliters of scintillation fluid was added and counted.

FIG. 2. The experiment was essentially the same as in Fig. 1, except ^{14}C -labeled carbodiimide ($\blacktriangle$) (2 mCi/mmole) was reacted with ^3H -labeled ($\square$) SV40 DNA I. ^{32}P -labeled SV40 DNA I ($\circ$) marker was cleaved in the same *Hin* D digest with ^{14}C -labeled CMC-modified ^3H -labeled SV40 DNA.

seen that no ^{14}C counts are observed (closed triangles) in any of the fragments, except A, B, and G. It is also apparent that the ^{14}C counts follow the ^3H counts in those fragments that react with CMC. It can be concluded from this experiment that A, B, and G are the only *Hin* D fragments that react with CMC. It is possible from the specific activity of ^{14}C -labeled CMC to calculate the amount of binding in the respective fragments. The total reactivity can be compared to the binding calculated from the buoyant shift measured in the preceding paper (15).

Binding analysis of [^{14}C]CMC to the *Hin* D A, B, and G fragments of SV40 DNA. Based on the specific activity of ^{14}C -labeled CMC and the amount of SV40 DNA used in the experiment, we can calculate the amount of binding to the respective fragments. The results are presented in Table 1. The tabulated reactivity for each fragment can be readily converted into a total value for the entire SV40 genome by multiplying the percentage of reactivity by the fractional molecular weight of each fragment and summing the total. This value is compared to the value obtained from the previous study (15) in which we employed the buoyant density shift produced by CMC and the partial specific vol-

TABLE 1. Percentage of reactivity of CMC to respective *Hin* D fragments and the number of CMC molecules bound per fragment

<i>Hin</i> D fragment and relative mol wt (% of SV40 DNA)	Reactivity with ^{14}C -labeled CMC (%)	No. of CMC mol bound
A 22.5	6.54	80
B 15.0	3.87	31
G 7.0	2.74	10
Totals in terms of base pairs	2.24% of SV40 DNA	121
Analysis by buoyant density shift and density of CMC from previous study (15)	2.0% of SV40 DNA	108

ume of the reagent. The reaction conditions were very similar except for the different sources of CMC. It can be seen that the results are in good agreement using two independent methods.

DISCUSSION

The results of the CMC binding analysis using the *Hin* D restriction endonuclease can be best examined in the context of previous results on the mapping of the single-strand-specific S_1

endonuclease (3), gene 32 protein, and the current results on the specificity of the initiation of transcription of *Escherichia coli* polymerase (14, 16, 30).

Figure 3 summarizes the data in the *Hin* D fragment map (6). It is apparent that there is excellent agreement between the binding of CMC to fragments A, B, and G and the sites cut by S_1 endonuclease on SV40 DNA I.

In the light of the above results, we can consider the possible structure-function relationships for the sensitive regions of superhelical SV40 DNA. The first question that is of particular significance is the structure of the regions containing unpaired bases in superhelical DNA. The previous two papers (15, 29) have presented evidence that can be interpreted with the model that supercoiling produces regions of

interrupted secondary structure. One can envision that different single-strand regions, produced by superhelical torsional forces, could migrate until they reached regions where sufficient intrastrand complementarity, i.e., formation of hairpin structures at these sites, would lower the free energy and localize the interrupted duplex structure. This localization of interrupted secondary structure is dependent on sequence relationships. Thus, SV40 and polyoma superhelical DNAs appear to have a limited number of localized sites for S_1 cleavage (3, 8) whereas ϕ X-RF is cleaved by *Neurospora crassa* endonuclease in a nonpreferential manner (K. Bartok and D. T. Denhardt, personal communication).

We suggest that some superhelical DNAs will contain localized hairpin regions which

FIG. 3. Map of the *Hin* D fragments of SV40 DNA with the location of the following sites: dark regions represent the area sensitive to the single-strand endonuclease S_1 (3); the *Hin* D fragments binding the indicated number of CMC molecules determined in this study. Gene 32 protein binding sites (22, 23) are represented by arrows inside the circle. Three pppAp initiation sites determined according to the methods of P. Lebowitz and Zain et al. (15, 17, 30; manuscript in preparation) used by *E. coli* polymerase (see text) are shown by the outside arrows with the direction of transcription. Early in vivo transcription is shown for comparison (13).

may show fluctuations from the most stable state (hairpin configurations). This introduces a more dynamic view which allows for the influence of environmental factors. Beard et al. (3) did observe that S_1 cutting shifted preferentially to 0.45 and 0.55 sites upon an increase in NaCl concentration from 75 to 250 mM. The additional consideration of sequence relationships suggest that some superhelical DNAs could have many transient intrastrand hairpins or single-strand sites and, consequently, many sites for single-strand nuclease attack. A test for this view would be the precise locations of CMC in order to sequence the regions reacted. Such a study is in progress.

Since gene 32 protein binds cooperatively to a single-strand DNA (26), it is striking that it binds only at one site, at 0.46, which corresponds to one of the three sites at which S_1 cleaves SV40 DNA, whereas it fails to bind to the B and G sites which are susceptible to S_1 cleavage. In addition, another gene 32 protein site has been discovered that does not map with S_1 or CMC sites (22). It has been pointed out (26) that the free energy of binding of a protein to a single strand of DNA (ΔG_{bind}), in a region normally double stranded under the prevailing environmental conditions, must at least offset the free energy favoring the native over the random coil conformation at that locus (ΔG_{conf}). These energetic considerations are applicable to the melting out of A-T-rich regions in duplex DNA, as well as the melting out of short hairpin regions. Both events are needed during replication (13, 26).

In the case of SV40 DNA I, it would be necessary that ΔG_{conf} or hairpins $< \Delta G_{\text{bind}}$ in order to melt out the proposed hairpin structures. The inability to satisfy favorable energetics for gene 32 protein melting of the 0.55 site in A and the sites in B and G fragments suggests stable hairpin sites at these locations in SV40 DNA I. Other structural features may play an important role. The cooperativity of hairpin melting might be partially controlled by protein-protein interactions, as bound gene 32 proteins interact favorably with one another (26). Steric factors in supercoiled DNA could affect the binding of gene 32 protein.

A variety of in vitro transcription studies have been performed on different superhelical DNAs (4, 5, 11, 14, 17, 18, 23, 24, 27, 28, 30, 31). Hayashi and Hayashi (10) showed that ϕ X-RF I is more rapidly transcribed than ϕ X-RF II. This kind of result has been obtained for superhelical λ (4) and PM2 DNA (23, 24, 27, 31). It was proposed that supercoiling increased nonspecific initiation (4, 27), since an increase in binding is more favorable in order to lower the free

energy by a coupled unwinding of the duplex structure and supercoils. However, if hairpin regions exist, then binding of RNA polymerase could occur at these sites. This would present easily recognizable, specific regions for the enzyme as well as promote more favorable energetics of binding.

A number of studies (11, 14, 17, 18, 24) suggest that the above model may reflect the enhanced transcription seen for superhelical DNA. Richardson (24) found that PM2 DNA I forms 16 stable binary complexes with *E. coli* RNA polymerase, whereas only 2 are found for closed, nonsupercoiled PM2 DNA. The reaction of PM2 DNA I modified with CMC, at a binding ratio of 0.01, eliminates 95% of the transcription capacity relative to untreated form I (M. Flashner and J. Lebowitz, unpublished data). This has also been found for SV40 DNA I (P. Hale and J. Lebowitz, unpublished data).

Recent studies (14, 16, 30) of the transcription of SV40 DNA with *E. coli* RNA polymerase show that there are three principal γ -[32 P]ATP initiation fragments: one, designated A_{II} , copied from the *Hin* D-G fragment, and the other two designated A_I and A_{III} , each copied from two separate sites in the *Hin*-A fragment (16, 30; P. Lebowitz, unpublished data). It was also reported that two additional pppAp and a limited number of pppGp sites (14) exist.

In Figure 3 we show the three pppAp initiation sites on the SV40 map. It can be seen that initiation occurs at 0.52 and 0.44 in the A fragment, and at 0.17 in the G fragment. The direction of *E. coli* RNA polymerase transcription is shown in Fig. 3, and it is completely asymmetric (28). The strand copied corresponds to that copied early in lytic infection and has been designated the (-) or early strand (12). An examination of Fig. 3 shows an extremely interesting structural feature: there is a potential hairpin site before each pppAp initiation site. Consequently, hairpin regions in these cases would promote considerable specificity in the interaction of *E. coli* polymerase with superhelical SV40 DNA. The formation of RNA polymerase promoter complexes would be achieved more readily at hairpin sites, since the energy needed to open the DNA for access to the template bases should be less than that for a fully paired structure (5). Hence SV40 DNA I could, in the pppAp initiation sites shown, bypass the activation step of opening an intact duplex strand. This would allow for more initiation events, which would explain the enhanced transcription of supercoiled DNA. The specificity of the RNA polymerase interaction would be dependent on whether open regions or hairpins can be fixed on the molecule. Further unwind-

ing by the formation of a DNA-RNA hybrid (14) would be assisted by the free energy decrease caused by a coupled unwinding of duplex and superhelical turns.

Recently, a series of papers was published on the *in vitro* transcription of SV40 DNA I, relative to the linear form, using calf thymus and rat liver RNA polymerases (11, 17, 18). These results are very similar to the ϕ X-RF and PM2 data and show enhanced transcription for SV40 DNA I. They conclude that unpaired bases are responsible for the enhanced rate due to an increased binding affinity for the RNA polymerase at unpaired sites (11). In contrast to *E. coli* polymerase, the mammalian RNA polymerases tested transcribed SV40 DNA I symmetrically. The incorporation of γ -[32 P]ATP and γ -[32 P]GTP suggests multiple initiation sites, although the number and location are not known (17). It is important to note that an initiation complex of the B RNA polymerase of calf thymus with SV40 DNA I apparently protects against S_1 cleavage (11).

An *in vivo* comparison of SV40 mRNA in the nucleus and the cytoplasm suggests that RNA synthesis occurs symmetrically and that anti-messsage is degraded prior to its transport to the cytoplasm (1, 12). This proposal agrees with the symmetrical synthesis observed *in vitro* for mammalian RNA polymerases. The hybridization pattern of cytoplasmic mRNA with *Hin* D fragments reveals that early message starts almost at the end of the A fragment (12) (Fig. 3). It is difficult at this time to reach conclusions solely from the hybridization data. First, we do not know the details of processing of viral mRNA and second, we know very little about the transcription of SV40 DNA complexes with histones (7, 9). It is entirely possible that structural variation will be produced by the formation of nucleosomes (7, 9). For example, a study of the mapping of the SV40-specific sequences transcribed *in vitro* from chromatin of SV40-transformed SV 3T3 cells shows that the early regions A through B (Fig. 3) were transcribed five to ten times more frequently than the remaining regions (2). In contrast, transcription of purified SV 3T3 DNA by *E. coli* polymerase produced equal frequencies of transcription from all regions of the integrated DNA (2).

This brief survey of aspects of transcription of SV40 clearly suggests that further experimental exploration is required in order to understand the structural relationship involved in transcriptional events for SV40 DNA I. We, along with other (3, 10, 11, 17, 18), suggest that there may be an important role played by regions of interrupted secondary structure form-

ing possible localized hairpins in the transcriptional process.

ACKNOWLEDGMENTS

J. L. was supported by Public Health Service Research Career Award KO4-CA00141-03 (formerly 5 KO4-CA07514) from the National Cancer Institute throughout the conduct and preparation of this study. J. L. thanks Syracuse University for the necessary sabbatical leave to carry out the reported research.

LITERATURE CITED

- Aloni, Y. 1974. Biogenesis and characterization of SV40 and polyoma RNAs in productively infected cells. Cold Spring Harbor Symp. Quant. Biol. 39:165-177.
- Astrin, S. M. 1975. Mapping of the SV40 specific sequences transcribed *in vitro* from chromatin of SV40 transformed cells. Biochemistry 14:2700-2711.
- Beard, P., J. F. Morrow, and P. Berg. 1973. Cleavage of circular, superhelical SV40 DNA to a linear-duplex by S_1 nuclease. J. Virol. 12:1303-1313.
- 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.
- Chamberlin, M. J. 1974. The selectivity of transcription. Annu. Rev. Biochem. 43:721-775.
- Danna, K. J., G. H. Sack, Jr., and D. Nathans. 1973. Studies of SV40 DNA VII. A cleavage map of the SV40 genome. J. Mol. Biol. 78:363-376.
- Germond, J.-E., B. Hirt, P. Oudet, M. Gross-Bellard, and P. Chambon. 1975. Folding of the DNA double helix in chromatin-like structures from simian virus 40. Proc. Natl. Acad. Sci. U.S.A. 72:1843-1847.
- Germond, J.-E., V. M. Vogt, and B. Hirt. 1974. Characterization of the single-strand-specific nuclease S_1 activity on double-stranded supercoiled polyoma DNA. Eur. J. Biochem. 43:591-600.
- Griffith, J. D. 1975. Chromatin structure: deduced from a minichromosome. Science 187:1202-1203.
- Hayashi, Y., and M. Hayashi. 1971. Template activities of the ϕ X-174 replicative, allomorphic deoxyribonucleic acids. Biochemistry 10:4212-4218.
- Hossenlopp, P., P. Oudet, and P. Chambon. 1974. Animal DNA-dependent RNA polymerases. Studies on the binding of mammalian RNA polymerases A1 and B to simian virus 40 DNA. Eur. J. Biochem. 41:397-411.
- Khoury, G., P. Howley, D. Nathans, and M. Martin. 1975. Posttranscriptional selection of simian virus 40-specific RNA. J. Virol. 15:433-437.
- Kornberg, A. 1974. DNA synthesis, p. 203-207. W. H. Freeman & Co., San Francisco.
- Lebowitz, P., and R. Bloodgood. 1975. Transcription of simian virus 40 DNA by *Escherichia coli* RNA polymerase: synthesis of a DNA-RNA hybrid and discrete RNAs under restrictive transcription conditions. J. Mol. Biol. 94:183-201.
- Lebowitz, J., C. F. Garon, M. Chen, and N. P. Salzman. 1976. Chemical modification of simian virus 40 DNA with a water-soluble carbodiimide. J. Virol. 18:205-210.
- Lebowitz, P., W. Seigel, and J. Sklar. 1974. *Hemophilus aegyptius* restriction endonucleases cleavage map of the simian virus 40 genome and its colinear relation with the *Hemophilus influenzae* cleavage map of SV40. J. Mol. Biol. 88:105-124.
- Mandel, J. L., and P. Chambon. 1974. Animal DNA-dependent RNA polymerases. Analysis of the RNAs transcribed *in vitro* from simian virus 40 superhelical DNA by

- mammalian RNA polymerases AI and B. Eur. J. Biochem. 41:379-395.
18. Mandel, J. L., 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 AI and B. Eur. J. Biochem. 41:367-378.
 19. Metz, D. H., and G. L. Brown. 1969. The investigation of nucleic acid secondary structure by means of chemical modification with a carbodiimide reagent. I. The reaction between N-cyclohexyl-N'- β -(4-methylmorpholinium) ethylcarbodiimide and model nucleotides. Biochemistry 6:2312-2328.
 20. 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.
 21. Morrow, J. F., and P. Berg. 1973. Location of the T4 gene 32 protein binding site on simian virus 40 DNA. J. Virol. 12:1631-1632.
 22. Reed, S. I., J. Ferguson, R. W. Davis, and G. R. Stark. 1975. T antigen binds to simian virus 40 DNA at the origin of replication. Proc. Natl. Acad. Sci. U.S.A. 72:1605-1609.
 23. Richardson, J. P. 1974. Effects of supercoiling on transcription from bacteriophage PM2 deoxyribonucleic acid. Biochemistry 13:3164-3169.
 24. Richardson, J. P. 1975. Initiation of transcription by *Escherichia coli* RNA polymerase from supercoiled and non-supercoiled bacteriophage PM2 DNA. J. Mol. Biol. 91:477-487.
 25. Smith, H. O., and K. W. Wilcox. 1970. A restriction enzyme from *Hemophilus influenzae*. I. Purification and general properties. J. Mol. Biol. 51:379-391.
 26. von Hippel, P. H., and J. D. McGhee. 1972. DNA-protein interactions. Annu. Rev. Biochem. 41:231-300.
 27. Wang, J. C. 1974. Interactions between twisted DNAs and enzymes: the effects of superhelical turns. J. Mol. Biol. 87:797-815.
 28. Westphal, H. 1970. SV40 DNA strand selection by *Escherichia coli* RNA polymerase. J. Mol. Biol. 50:407-420.
 29. 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.
 30. Zain, B. S., R. Dhar, S. M. Weissman, P. Lebowitz, and A. M. Lewis. 1972. Preferred site for initiation of RNA transcription by *Escherichia coli* RNA polymerase within the simian virus 40 DNA segment of the nondefective adenovirus-simian virus 40 hybrid viruses AD2⁺ND₁ and AD2⁺ND₃. J. Virol. 11:682-693.
 31. 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.