RNA POLYMERASE–PROMOTOR COMPLEX STABILITY ON SUPERCOILED AND RELAXED DNA

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1. Introduction

When covalently closed circular DNAs are extracted from cells, they are usually recovered as superhelical DNAs [1]. The superhelical turns can be removed from such DNAs by treatment with one of the several 'DNA relaxing proteins' to yield a 'relaxed' covalently closed circular DNA [2–4]. These two classes of DNA, superhelical and relaxed, frequently differ in their behaviour as enzyme substrates [5]. The enzyme RNA polymerase transcribes superhelical PM2 DNA more efficiently than relaxed PM2 DNA [5]. With DNA as a template, the efficiency of transcription by RNA polymerase increases with increasing negative superhelicity [6]. One of the early stages in transcription, the polymerase–promoter complex, has been shown to be more stable on superhelical PM2 DNA than on relaxed PM2 DNA [7].

None of the work cited above has directly quantitated the differences in stability of the promoter complexes formed on superhelical versus relaxed DNAs. The following experiments provide a direct measurement of the differences using the nitrocellulose filter binding assay [8]. We used SV40 DNA for the investigation because it seems to have only one major promoter site for *Escherichia coli* RNA polymerase [9,10]. We standardized the results for SV40 to those of fd RF because the polymerase interactions in this system have been more thoroughly investigated [11].

Abbreviations: Throughout this paper RNA polymerase indicates the holoenzyme from *Escherichia coli* (EC 2.7.7.6 nucleosidetriphosphate: RNA nucleotidyl transferase), fd RFI° the relaxed form of the phage fd replicative form DNA, SV40 FoI the superhelical double-stranded form of SV40 DNA, SV40 FoI° the relaxed double-stranded form of SV40 DNA

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2. Materials and methods

E. coli RNA polymerase was prepared according to Burgess [12] through the ammonium sulfate fractionation. The enzyme was then chromatographed on DNA agarose [13] and finally on Biogel Agarose 1.5 m. DNA relaxing enzyme from Hela cells was a gift from W. Keller, Cold Spring Harbor, NY. This enzyme was used to prepare SV40 FoI° [4]. The SV40 FoI DNA was a generous gift from P. Gruss, German Cancer Research Center, Heidelberg, FRG. The fd RFI° was prepared from single-stranded fd DNA by repair synthesis by *E. coli* DNA polymerase I and *E. coli* DNA ligase [14,6]. The filter binding assays were performed as described [15]. Stabilizing triphosphates were present at concentrations of 10 μM.

3. Results

The relative rates of binding of RNA polymerase to fd RFI° versus SV40 FoI were determined by titrating a competing mixture of the two DNAs with the enzyme. At low molar ratio of enzyme to DNA all enzyme is bound in stable promoter complexes

and no significant dissociation of these complexes occurs during the short period of competition with unlabelled DNA at 120 mM KCl (data not shown and [15,19]). Therefore the ratio of the two complexed DNA species in the filter binding assay reflects the differences in the relative rates of promoter selection between these DNAs. Under the conditions used (time and ionic strength) one of the five major fd promoters is selected for to approximately 85% [15,16]. If SV40 FoI DNA is included into the experiment this DNA is complexed preferentially at low enzyme/DNA ratios (fig.1A), indicating a higher rate of binding of the enzyme to the supercoiled SV40 DNA than to relaxed fd RF DNA.

The preferential binding of RNA polymerase to SV40 DNA, while reduced, persists, even when the latter is in a relaxed configuration. This is demonstrated by a competition between fd RFI° and SV40 FoI° for RNA polymerase (fig.1B). A quantitative estimation of the above findings indicates a preference of RNA polymerase for the SV40 promoter over the strongest fd promoter by a factor of 2, and an additional three-fold preference for promoters in the superhelical state.

As a control for correct binding it was tested whether the promoter complexes were labile to a cold-shift, but stabilized against decay by preincubation with the initiating ribonucleosidetriphosphate. Selective stabilization of either promoter is expected, since RNA synthesis starts with an oligo-G run at the fd promoter [15], and with an oligo-A run at the SV40 promoter [17]. The results obtained figs.1A and 1 B agree with these predictions, except for the cold-shift experiment with the superhelical SV40 DNA which showed incomplete dissociation of the promoter-bound enzyme after 60 min at 0°C. This suggests an unusually high stability of the promoter complexes in superhelical DNA.

The differences in complex stability due to supercoiling were investigated further by comparing the rates of enzyme release from the SV40 promoter in the relaxed or in the supercoiled state using fd RFI°

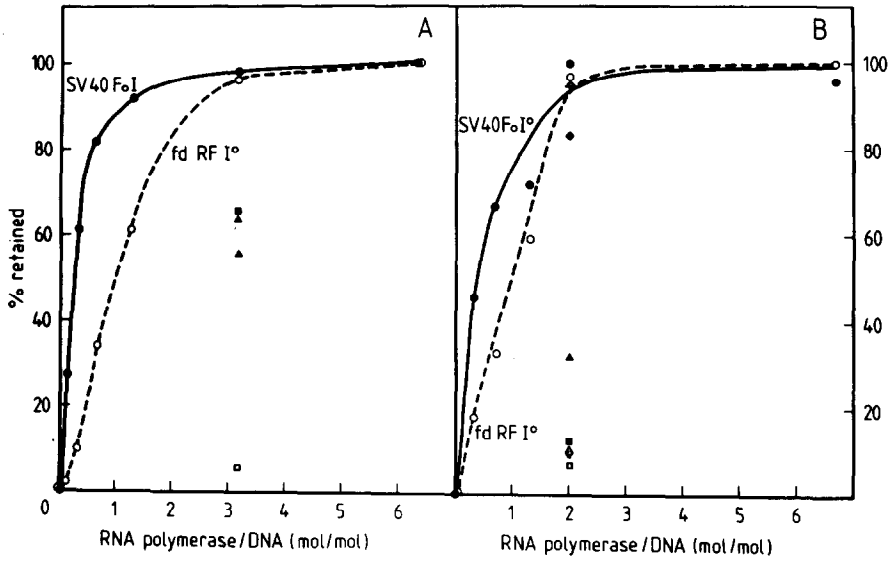

Fig.1. Polymerase–promoter complex formation. Each sample contained 0.45 pmol of ^{32}P-labeled fd RFI°, 0.04 pmol of ^{3}H-labeled SV40 FoI DNA and RNA polymerase to give the molar ratios indicated. Complexes were formed for 10 min at 37°C in 120 mM KCl. Denatured calf thymus DNA was added to a concentration of 300 μg/ml and after a further 10 min at 37°C the samples were diluted to 400 μl and filtered. (A) (○) ^{32}P-labeled fd RFI° complexes, (●) ^{3}H-labeled SV40 FoI complexes. (□) RFI° complex remaining after 60 min at 0°C, (△) the corresponding value in the presence of 10 mM rGTP, (■) SV40 FoI complex remaining after 60 min at 0°C, (▲) the corresponding value in the presence of 10 mM rGTP. (B) Symbols as described under (A) except for (●) ^{3}H-labeled SV40 FoI°. (◆) ^{3}H-labeled SV40 FoI° complex, stabilized by rATP, (◇) is the ^{14}C-labeled fd RFI° complex stabilized by rATP.

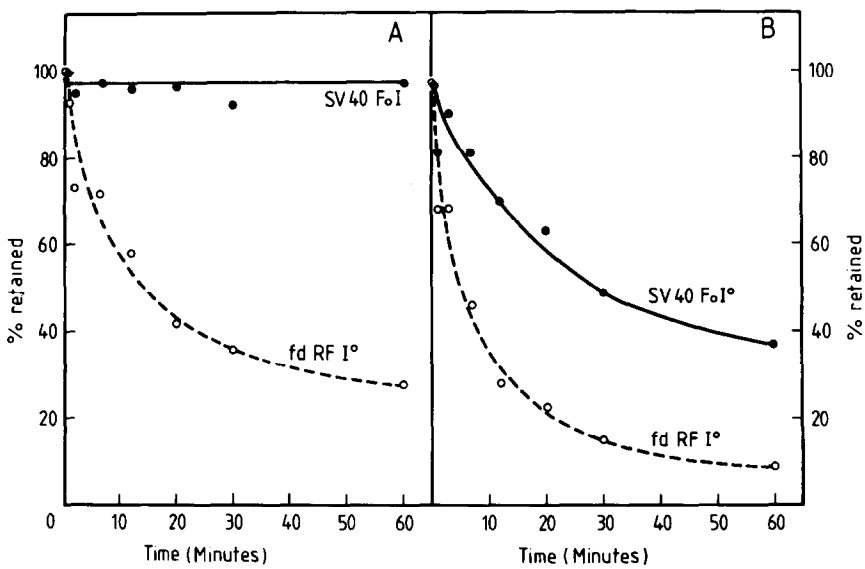

Fig.2. Kinetics of polymerase–promoter complex decay. Complexes were formed at a polymerase/DNA ratio of 3 in 120 mM KCl. Denatured calf thymus DNA was added to 300 μg/ml and after 1 min the KCl concentration increased to (A) 170 mM or (B) 190 mM. (○) ^{32}P-labeled fd RFI° complex remaining, (●) ^{3}H-labeled SV40 FoI complex (A) or ^{3}H-labeled SV40 FoI° complex (B).

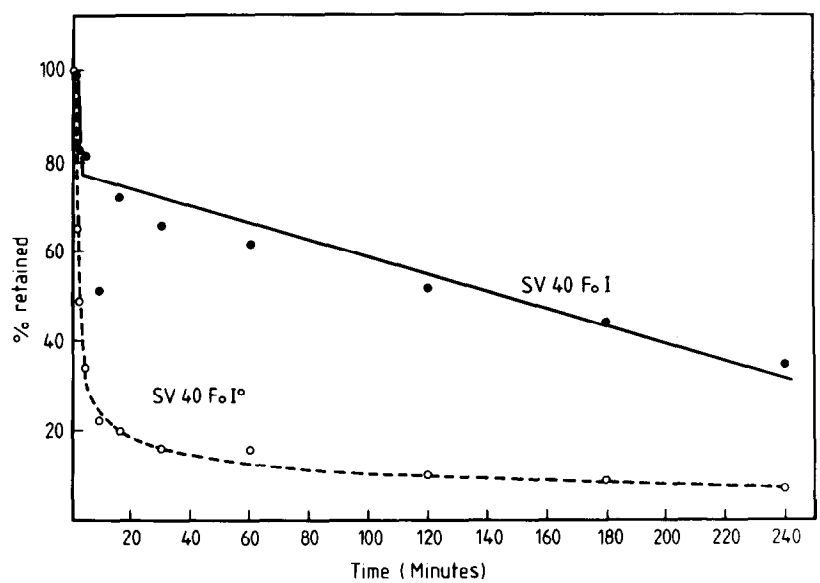

Fig.3. Kinetics of polymerase–promoter complex decay. Complexes were formed as described in fig.2. The KCl concentration was increased to 240 mM and the complex decay was measured. (●) ^{3}H-labeled SV40 FoI° complexes, (○) ^{32}P-labeled SV40 FoI complexes.

as a standard. No significant dissociation of polymerase from both SV40 FoI and fd RFI° was detected within 30 min at 120 mM KCl ([16] and unpublished data). If the ionic strength was increased to 170 mM KCl the fd RFI°–polymerase complex decayed with a half-time of approximately 15 min, but the corresponding SV40 FoI complex did not decay noticeably after 60 min (fig.2A). Figure 2B shows a comparison of the decay rates of fd RFI° complexes with SV40 FoI° complexes. Again, even with the relaxed SV40 DNA, the SV40 complex is more stable than the fd complex (half-times of 22 min and 7 min, respectively). The greatly increased stability observed when using superhelical SV40 DNA, is however, no longer evident. Further studies with the SV40 FoI–polymerase complexes (data not shown) showed that they have a half-time of 16 min in 480 mM KCl.

Quantitating the differences in polymerase complex stabilities more directly was complicated by the extreme differences between relaxed and superhelical DNA complexes. The SV40 FoI° complex decays rapidly at KCl concentrations of more than 200 mM, and the SV40 FoI complex decays very slowly at KCl concentrations of less than 300 mM KCl. Since the rates of decay are substantially different, we found it difficult to make accurate measurements. The magnitude of the differences we observed were 100–150-fold. One of several attempts to quantitate this difference is shown in fig.3. In this experiment, at 240 mM KCl, the superhelical DNA–polymerase complex decayed with an extrapolated half-time of 210 min, whereas the relaxed DNA–polymerase complex decayed with half-life of 2 min, giving a value of 105-fold for the difference in complex stability.

4. Discussion

Promoter–polymerase complexes are usually described as being sensitive to high salt and low temperature [11,18]. Our results indicate that the superhelical DNA–RNA polymerase complexes are about 100-fold more stable to these conditions and also form faster than relaxed DNA–RNA polymerase complexes. Qualitatively similar effects of supercoiling on RNA polymerase–promoter interaction have been observed in an analogous set of experiments with fd RF DNA [19] and in experiments with PM2 DNA using transcription to assay for heparin-resistant promoter complexes [7]. They are consistent with the concept that the formation of promoter complexes requires local unwinding of base-pairs in the promoter DNA, which is energetically favoured in the superhelical state.

Under the conditions used (low enzyme/DNA ratio) 0.12 M KCl, we have been studying primarily the interaction of RNA polymerase with the major SV40 promoter (map position 0.17) which directs the synthesis of assymetric in vitro c-RNA starting with an oligo A-run [17]. This is indicated by the fact that essentially all promoter complexes formed at low enzyme/DNA ratio could be stabilized against dissociation by oligo-A synthesis (fig.1 and unpublished results). Furthermore these complexes were found to be located exclusively in restriction fragment Hae III-C (map position 0.11–0.21) in both superhelical and relaxed SV40 DNA (unpublished results). The presence of (an) additional minor promoter site(s) is suggested by the variable but significant fraction (20–40%) of promoter complexes that could be stabilized by oligo-G synthesis.

The interactions of the fd promotors with RNA polymerase have been described [16,19]. The conditions used in the present study allow us to compare one of the fd promoters (that in map position 0.94) to the SV40 promoter. Both of these promoter regions have been sequenced and the two are identical in 6 out of 7 of the bases of the proposed 'binding sequence' [20] and also show additional sequence homology in a second non-transcribed recognition sequence [19]. Before functions can be correlated to these sequences, more such studies are needed, expecially comparisons using the lac UV 5 promoter, the binding sequence of which is identical to that of the SV40 promoter [20].

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References

[1] Helinski, D. R. and Clewell, D. B. (1971) Ann. Rev. Biochem. 40, 899–942.

[2] Wang, J. C. (1971) J. Mol. Biol. 55, 523–533.
[3] Champoux, J. J. and Dulbecco, R. (1972) Proc. Natl. Acad. Sci. USA 69, 143–146.
[4] Keller, W. (1975) Proc. Natl. Acad. Sci. USA 72, 2550–2554.
[5] Wang, J. C. (1974) J. Mol. Biol. 87, 797–816.
[6] Botchan, P., Wang, J. C. and Echols, H. (1973) Proc. Natl. Acad. Sci. USA 70, 3077–3081.
[7] Richardson, J. P. (1975) J. Mol. Biol. 91, 477–487.
[8] Hinkle, D. C. and Chamberlin, M. J. (1972) J. Mol. Biol. 70, 157–185.
[9] Westphal, H. (1970) J. Mol. Biol. 50, 407–420.
[10] Allet, B., Roberts, R. J., Gestland, R. F. and Solem, R. (1974) Nature 249, 217–226.
[11] Seeburg, H. P. and Schaller, H. (1975) J. Mol. Biol. 92, 261–277.
[12] Burgess, R. R. and Jendrisak, J. J. (1975) Biochemistry 14, 4634–4638.
[13] Nüsslein, C. and Heyden, B. (1972) Biochem. Biophys. Res. Commun. 47, 282–289.
[14] Heyden, B. (1974) Ph. D. thesis, University of Tübingen, FRG.
[15] Nüsslein, C. and Schaller, H. (1975) Eur. J. Biochem. 56, 563–569.
[16] Heyden, B., Nüsslein, C. and Schaller, H. (1972) Nature New Biol. 240, 9–12.
[17] Dhar, R., Zain, S., Weissman, S. M., Pan, J. and Subramanian, K. (1974) Proc. Natl. Acad. Sci. USA 71, 371–375.
[18] Chamberlin, M. (1974) Ann. Rev. Biochem. 43, 721–775.
[19] Seeburg, H. P., Nüsslein, C. and Schaller, H. (1977) Eur. J. Biochem. in press.
[20] Pribnow, D. (1975) J. Mol. Biol. 99, 419–443.