INFLUENCE OF SALT ON TRANSCRIPTION BY T_4 CORE RNA POLYMERASE

Ruth Kirsten KLEPPE
Department of Biochemistry, University of Bergen, Bergen, Norway

Received 10 January 1975

1. Introduction

T_4 phage infection of *E. coli* results in numerous changes in the transcription process in the cell such as cessation of host RNA synthesis and the production of many new classes of viral RNAs [1,2]. During the infection process the host RNA polymerase also becomes modified in both α and β subunits. The changes in the α-subunit involves incorporation of one adenylate residue [3,4] whereas β and β' subunits are subjected to changes in the amino acid composition as detected by tryptic analyses [5]. Recently also some small molecular weight phage coded proteins have been shown to be associated with the enzyme [6,7]. With regard to the σ subunit varying amounts of this subunit have been reported to be present in the enzyme. Thus Seifert et al. [8] found that the enzyme isolated immediately after infection contained some σ subunit whereas the enzyme isolated late in the infection period contained no σ subunit. Similar findings have also been reported by Bautz and Dunn [9]. Travers [10] and also Khesin [11] have, however, noted that the enzyme contains considerable amounts of σ subunits. More recently Stevens [12] has shown that loss of σ subunit may occur during the purification procedure.

The transcription properties of T_4 RNA polymerase have been studied in many laboratories and some disagreements have been noted. Some of the differences reported presumably could be due to variation in the σ subunit content. Another factor which also may be of importance in this respect is the role of ionic strength in transcription by T_4 RNA polymerase. It has recently been shown [12,13] that salt inhibits T_4 RNA polymerase and in many of the studies reported different ionic strengths have been used. It would therefore seem important to have a thorough knowledge of the influence of ionic strength on both T_4 core and holo RNA polymerase. The present work deals with the effect of KCl on transcription properties of T_4 core RNA polymerase. A comparison with core and holo RNA polymerase from uninfected *E. coli* is also described.

2. Materials and methods

2.1. *Preparation of RNA polymerases*

T_4 RNA polymerase was prepared from E. coli B 62 cells infected with T_4 am N82 at a m.o.i. of 8 and harvested 30 min after infection. The procedure followed was modified somewhat from that of Chamberlin and Berg [14]. The modification consisted of inclusion of a gel filtration step, i.e. a column of agarose 1.5 M (90 X 2.5 cm) equilibrated with 50 mM Tris pH 8.0, 1 mM dithioerytritol and 0.3 M $(NH_4)_2SO_4$ prior to chromatography on the DEAE cellulose column. The specific activity of the enzyme was approximately 2000 units/mg protein using calf thymus DNA as a substrate. The purity of the enzyme was analyzed by SDS gel electrophoresis. The T_4 RNA polymerase obtained from the DEAE cellulose column contained no σ subunit. In most of the preparations, however, a subunit with mol. wt of approx. 60 000 was found. RNA polymerase prepared from *E. coli* B 62 by the same procedure gave the normal amount of σ subunit and in this case the subunit with mol. wt of approx. 60 000 was never observed. The role of the latter protein species is unknown. It could, however, be separated from the T_4 RNA polymerase by centrifugation in glycerol gradient in the presence of 0.2 M KCl, fig.1, or by chromatography on a column of phosphocellulose. In most experiments the latter

Fig.1. SDS-polyacrylamide gel electrophoresis of RNA polymerase from T_4 amN82 infected *E. coli* B 62 after the glycerol gradient centrifugation step.

enzyme was used. However, no difference could be detected between the two enzyme species with regard to inhibition by salt.

Core RNA polymerase from uninfected cells was prepared from the holoenzyme as described by Burgess [17].

2.2. *DNAs*

DNA from *E. coli* B and T_4 were prepared by phenol extraction (15,16). The T_4 phages were treated with DNase prior to centrifugation in CsCl gradient. Calf thymus DNA and poly [d(A–T) · d(A–Y)] were purchased from the Sigma Chemical Company. The synthetic DNAs used were made in the laboratory of Dr H. G. Khorana using procedures developed in this laboratory essentially as described in [18].

2.3. *Ribonucleoside-5triphosphates and other chemicals*

Unlabelled ribonucleoside-5-triphosphates were obtained from P.L. Biochemicals and Sigma Chemical Company. [^{3}H]- and [^{14}C]- labelled ribonucleoside-5-triphosphates were from Schwartz Biochemicals and Amersham Radiochemicals. The compounds were checked for purity by paperchromatography in solvent systems previously described [19].

Rifampicin was obtained from Mann Research Laboratories and streptolydigin was a gift from Dr B. Weissblum, Madison, USA.

2.4. *Enzyme assay*

The standard RNA polymerase assay system contained 40 mM Tris–HCl pH 8.0, 8 mM $MgCl_2$, 12 mM β-mercaptoethanol, 0.3 mM each of the four ribonucleoside triphophates, one of them being radioactively labelled with a specific activity of 2000–4000 cpm/nmol and 200 μg calf thymus DNA/ml. The reaction temperature was 37°C and the incubation period 5 min. The radioactive RNA was precipitated on filter paper discs which were then subsequently washed 4 times with cold 5% trichloroacetic acid, then once with ether : ethanol (1:1) and then with ether. The filters were then counted in a liquid scintillation counter.

2.5. *Determination of proteins*

Protein was determined according to the method of Lowry et al. [20].

2.6. *SDS gel electrophoresis*

This was carried out as previously described [21].

3. Results and discussion

3.1. *Influence of salt on transcription*

Addition of KCl to the reaction mixtures proved to have a marked effect on the RNA synthesis catalyzed by T_4 core RNA polymerase as illustrated in fig.2. With T_4 DNA as a template increasing concentrations of KCl as expected gave an increase in the rate of RNA synthesis with RNA polymerase from uninfected

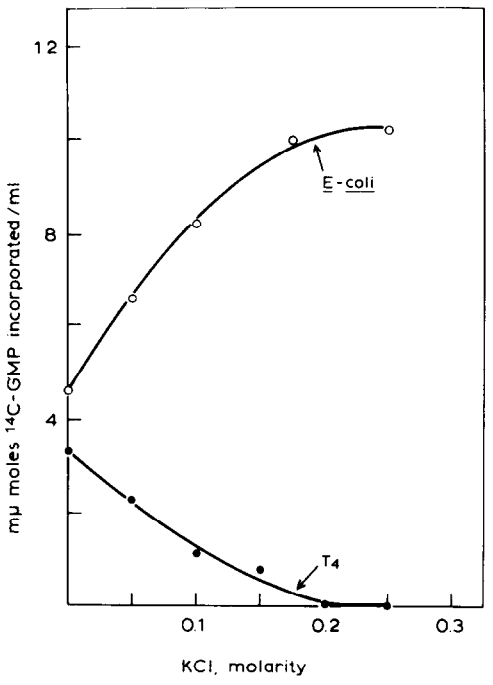

Fig.2. Influence of different concentrations of KCl on transcription of T_4 DNA by RNA polymerase, holo enzyme, from *E. coli* B 62 and T_4 core RNA polymerase. The assays were carried out as described in Methods.

E. coli B whereas with the enzyme from T_4 infected cells the synthesis decreased and at a concentration of 0.2 virtually no RNA synthesis was observed. The time-course of transcription of T_4 and calf thymus DNA in the presence and absence of 0.2 M KCl for the three different RNA polymerase species is shown in fig.3. With calf thymus DNA as a template salt had little influence on the rate of transcription by T_4 core RNA polymerase. In the case of the holo enzyme from uninfected *E. coli* B, as expected, a large increase in the rate of transcription in the presence of salt was seen both with T_4 and calf thymus DNA. With the core polymerase salt also increased the rate of transcription but not to the same extent as for the holo enzyme. Thus T_4 core RNA polymerase clearly possesses different transcriptional properties from those of the enzymes from uninfected cells.

3.2. *Effect of salt on the initiation and elongation process*

The mechanism of inhibition of RNA synthesis by salt was investigated in more details using T_4 DNA as a template. The data presented in fig.4, top part, show that when KCl was added after initiation had taken place no inhibition was observed. On the contrary a slight stimulation was seen. For comparison the data

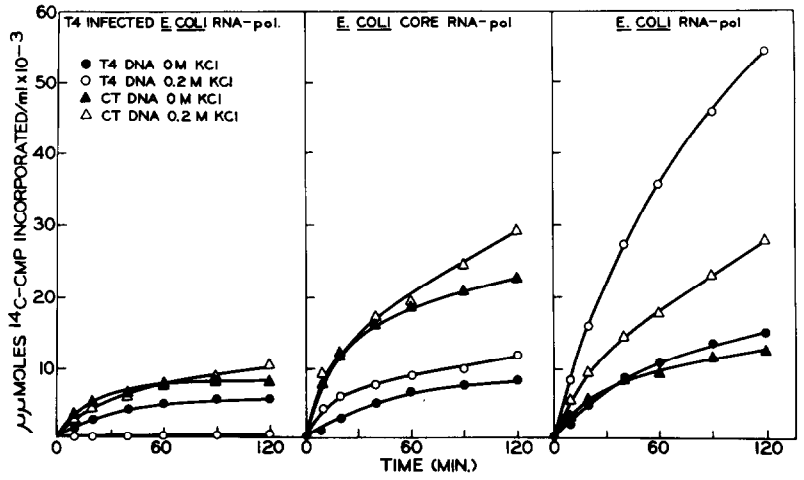

Fig.3. Time course of transcription of T_4 and calf thymus DNA in the presence and absence of 0.2 M KCl. Comparison between T_4 core RNA polymerase and core and holo enzyme from uninfected cells. The concentrations of T_4-DNA and CT-DNA were 50 μg/ml. The total reaction volume was 100 μl and aliquots of 15 μl were withdrawn for TCA precipitation. The protein concentration used was 50 μg/ml;
(●–●) T4-DNA, no KCl; (○–○) T4-DNA, 0.2 M KCl; (▲–▲) CT-DNA, no KCl; (△–△) CT-DNA, 0.2 M KCl.

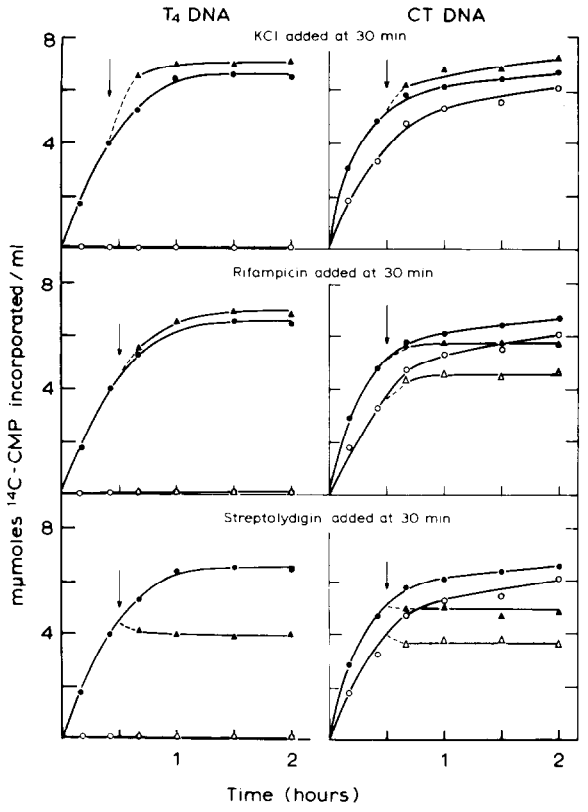

Fig.4. Influence of KCl on initiation and elongation process. T_4-DNA and CT-DNA, both at 30 μg/ml, were transcribed by T_4 RNA polymerase (48 μg/ml) at standard conditions without KCl (closed symbols) or in the presence of 0.2 M KCl (open symbols). The arrows indicate addition of either KCl, rifampicin or streptolydigin after 30 min of reaction at 37°C (triangles). The top figures show addition of KCl to a final concentration of 0.2 M. The middle figures show the effect of adding rifampicin to a final concentration of 2×10^{-5} M and the bottom figures show the effect of adding streptolydigin to a final concentration of 2×10^{-4} M. The reaction volumes were 100 μl and aliquotes of 15 μl were withdrawn for TCA precipitation. The reaction was followed by incorporation of ^{14}C-CMP of specific activity of 2470 cpm/nmol.

obtained with calf thymus DNA is also given. Rifampicin did not inhibit RNA synthesis in the presence of salt when this was added after initiation had occurred, fig.4, middle part. Streptolydigin on the other hand inhibited prolongation of RNA chains both with T_4 and calf thymus DNA as templates, fig.4, botton part. These data therefore suggest that the initiation process on T_4 DNA by T_4 core RNA polymerase is particularly sensitive to ionic strength.

When T_4 core RNA polymerase was mixed with

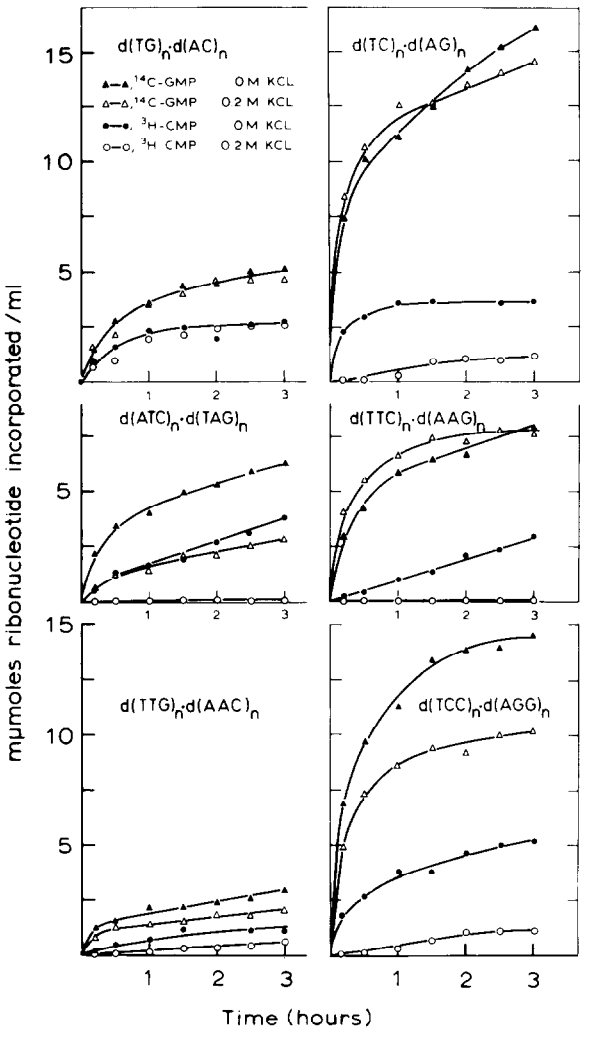

Fig.5. Transcription of synthetic DNAs. Reaction volumes were 100 μl and 10 μl were withdrawn at various times as shown in the figure. The concentration of T4 RNA polymerase was 48 μg/ml and the concentration of the synthetic DNAs was 0.4–0.5 A_{260}/ml. The total amount of nucleoside-5′-triphosphates was 1.6 mM, the ratio between the four triphosphates corresponding to the ratio between the bases of the DNA used as template. The reactions were followed by ^{3}H-CMP and ^{14}C-GMP incorporation into 5% TCA precipitable material, the specific activity being 1140 cpm/nmol and 2680 cpm/nmol. Each DNA was transcribed in the presence and absence of 0.2 M KCl.

[^{32}P]-PP_i, ATP and T_4 DNA it was found that pyrophosphate exchange did take place under conditions where no RNA synthesis was detectable, i.e. in the presence of 0.2 M KCl (data not shown). Thus it would appear that the enzyme is capable of binding to T_4 DNA in the presence of salt.

3.3. *Transcription of synthetic DNAs*

The results above suggested that the inhibition of transcription by salt was highly dependent on the nature of the DNA employed. In order to elucidate this problem further the transcription of six different synthetic DNAs of copolymeric structure was studied and the results are shown in fig.5. The three DNAs on the right hand side of this figure contain only purines in one strand and pyrimidines in the other, whereas the DNAs in the left hand part of the figure have purines and pyrimidines in both strands. The RNA synthesis catalyzed by T_4 core RNA polymerase was followed by double radioactive labelling, chosen in such a way that transcription of both strands in a given DNA could be followed simultaneously. All templates were transcribed both in the presence and absence of 0.2 M KCl. In general the following conclusions could be drawn from these experiments: There was a marked strand selectivity with regard to transcription, i.e. pyrimidine rich strands were transcribed at a higher rate than purine rich. In the presence of 0.2 M KCl the transcription of purine rich strands in most cases was almost completely inhibited whereas transcription of pyrimidine rich strands was mostly unaffected by salt. One of the DNAs used, poly $d(TG)_n \cdot d(AC)_n$, contains equal amount of purines and pyrimidines in both strands and in this case, as expected, salt had no influence on transcription of either strand.

With regard to initiation and assymetry of transcription of synthetic DNAs in the absence of salt T_4 core RNA polymerase resembles the holo enzyme from uninfected cells [18]. The inhibition pattern obtained with T_4 DNA in the presence of salt is similar to that of the purine rich strands of the synthetic DNAs. It is tempting, therefore, to suggest that the T_4 core enzyme in the presence of salt preferably binds to purine rich sites on the T_4 DNA.

Acknowledgements

This study was supported financially by the Nansen Foundation and by the Norwegian Research Council for Science and Humanities. I also wish to thank Dr H. G. Khorana for generous support and encouragement.

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