# Effect of salt on the transcription of T7 DNA by RNA polymerase from T4 phage-infected E.coli*

Audrey Stevens

Biology Division, Oak Ridge National Laboratory, P.O.Box Y, Oak Ridge, TN 37830, USA

Received 14 January 1977

## ABSTRACT

Transcription of T7 DNA by T4 core enzyme with host sigma is more sensitive to KCl than that by host core enzyme with host sigma. When salt is added after initiation of RNA chains has occurred, it is not inhibitory. Salt affects the binding of T4 enzyme to T7 DNA to the same degree as the binding of host enzyme. Active preinitiation complex formation is inhibited more by salt with the T4 enzyme and the inhibition is temperature-dependent.

## INTRODUCTION

Transcription of T4 DNA by RNA polymerase from T4 phage-infected cells has been shown to be salt-sensitive[1]. Part of the salt-sensitivity of T4 holoenzyme has been shown to be due to the presence of a salt-promoted inhibitor of transcription[2,3]. The inhibitor is removed along with the sigma subunit on phosphocellulose chromatography. T4 core enzyme resulting from the phosphocellulose chromatography is also salt sensitive[1,4]. This paper deals with the site of inhibition of salt on the core enzyme. The site of inhibition has been studied by coupling the T4 core enzyme with host sigma so that preinitiation complex formation can be more easily measured. (Host sigma was used because of greater availability. The sigma subunit from T4-infected cells is similar after removal of the above described inhibitor[2].) When T7 DNA is used as a template with the T4 enzyme the reaction is much more sensitive to salt than when host core enzyme and host sigma are used. Results on the effects of salt on DNA-enzyme binding, on active preinitiation complex formation, as well as on the overall reaction are presented here.

## MATERIALS AND METHODS

Host core enzyme and host sigma were prepared as described previously[1]. T4 core

*This investigation was supported by the Energy Research and Development Administration under contract with the Union Carbide Corporation.

enzyme was prepared from phage T4 am 47⁻ X 42⁻-infected E. coli as previously described[1]. T7 DNA was isolated from T7 phage according to the procedure of Thomas and Abelson[5].

For assay of RNA polymerase, the reaction mixtures (0.2 ml) contained [$^{14}$C]ATP (Schwarz/Mann) (0.25 mM, specific activity, 3400 cpm/nmol), UTP, CTP, and GTP (P-L Biochemicals, Inc.) (0.25 mM each), Tris buffer (20 mM, pH 7.8), $MgCl_2$ (10 mM), bovine serum albumin (Schwarz/Mann) (100 µg), 2-mercaptoethanol (10 mM), T7 DNA (10 µg), and enzyme. Incubation was for 10 min at 37° and determination of radioactivity incorporated into RNA was carried out as previously described[6].

The assay of RNA polymerase binding to T7 DNA was essentially as described by Mueller[7]. The reaction mixtures (0.2 ml) contained Tris buffer (20 mM, pH 7.8), $MgCl_2$ (10 mM), 2-mercaptoethanol (10 mM), bovine serum albumin (100 µg), T7 DNA (10 µg), and enzyme. Control reaction mixtures lacked T7 DNA. After incubation for 10 min at 37°C, 3.9 µg of poly (dA-dT) (Miles Laboratories), 50 nmoles of [$^{14}$C]-ATP (specific activity, 4000 cpm/nmole) and 50 nmoles of UTP were added. The reaction was continued at 37°C for another 10 min and determination of radioactivity incorporated into acid-insoluble material was carried out as described previously[6]. The reactivity of poly (dA-dT) as compared to the control measured the amount of unbound enzyme.

For assay of preinitiation complex formation, reaction mixtures contained the components described above for the binding reaction mixtures. After incubation for 15 min at a given temperature, 2 µg of rifampicin (Calbiochem), 50 nmoles of [$^{14}$C]ATP (specific activity, 8000 cpm/nmole), and 50 nmoles each of UTP, CTP, and GTP were simultaneously added. The reaction was continued at 37°C for another 5 min, and determination of radioactivity incorporated into RNA was carried out as previously described[6].

## RESULTS AND DISCUSSION

Figure 1 shows the effect of KCl concentration on the overall synthesis reactions of T4 enzyme and host enzyme with T7 DNA as a template. The overall reaction with T4 enzyme is stimulated by low concentrations of KCl (0.05 M and 0.1 M), but is inhibited by concentrations higher than 0.1 M. The reaction with host enzyme is stimulated by KCl concentrations up to 0.2 M and inhibited by 0.3 M. (Similar results with host enzyme have been reported previously[8].)

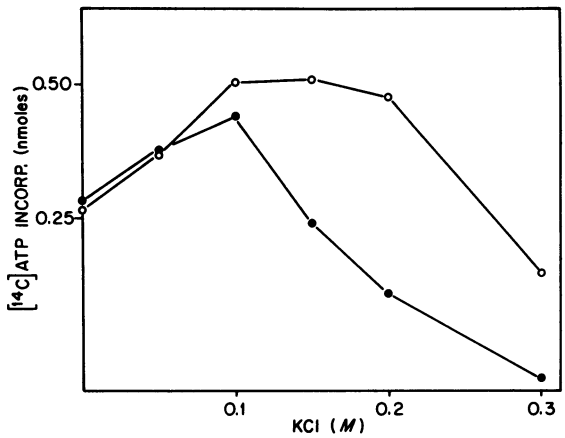


Figure 1. Effect of KCl concentration on the reactions of T4 enzyme and host enzyme with T7 DNA as a template. Reaction mixtures were as described under Materials and Methods with the indicated amount of KCl. One μg of T4 core enzyme and 0.75 μg of host sigma were used (●), or 1 μg of host core enzyme and 0.75 μg of host sigma (O).

To find out whether any of the effect of KCl on the T4 enzyme was at the stage of elongation or termination of RNA chains, the effect of KCl on RNA synthesis which had been initiated by incubation with three ribonucleoside triphosphates was studied. The complete reaction system, minus UTP, was incubated for 10 min at 37°C and then UTP and KCl were added. The results are shown in Table I. A slight stimulatory effect of KCl was found at both 0.1 M and 0.2 M. The results showed no inhibitory effect of KCl on elongation or termination of RNA chains and are similar to those reported for host enzyme with T7 DNA[8].

The effect of KCl on the binding of both enzymes to T7 DNA was studied by the competing template method of Mueller[7]. Results on the amounts of T4 enzyme and host enzyme bound in the absence of added KCl, and with 0.1 M and 0.2 M KCl, are shown in Figure 2. The host enzyme binds slightly better than the T4 enzyme in the absence of KCl, but the binding of both is decreased about 50% by 0.2 M KCl. The similar results obtained with the two enzymes suggests that the greater sensitivity to KCl of the T4 enzyme is not at the stage of nonspecific binding.

Mangel and Chamberlin[9] and Domingo et al.[10] have studied the effect of ionic strength on the reactions of host enzyme with T7 DNA and with Azotobacter phage A21 DNA respectively. They found that a salt-dependent equilibrium exists between an inactive polymerase-DNA complex and an active preinitiation complex (called "closed"

Table I. Effect of KCl concentration on RNA synthesis in reaction mixtures having a prior incubation minus UTP*

| KCl concentration (M) | [$^{14}$C]ATP incorporated (nmoles) |
|---|---|
| None | 0.41 |
| 0.1 | 0.49 |
| 0.2 | 0.50 |

*The initial reaction mixtures contained the components described for the assay of RNA polymerase reactions in Materials and Methods, but lacked UTP. One µg of T4 core enzyme and 0.75 µg of host sigma were used. After a 10-min incubation period at 37°C, KCl and 50 nmoles of UTP were added. The reaction was continued for another 10 min at 37°C; then it was stopped and radioactivity incorporated into RNA was determined as described previously[6].

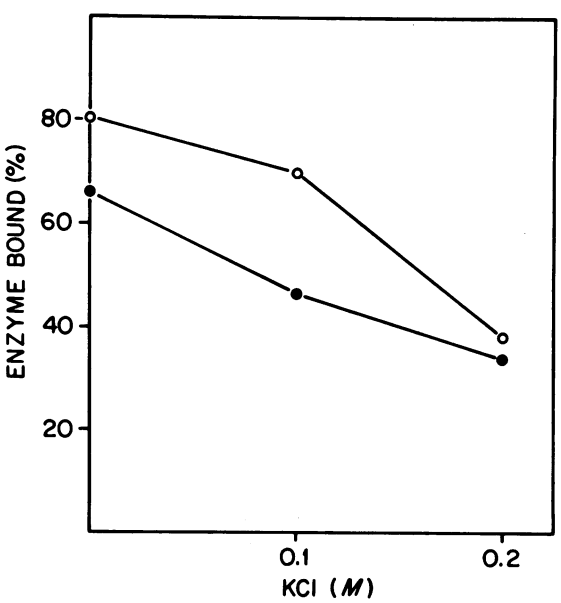


Figure 2. Effect of KCl on the binding of T4 enzyme and host enzyme to T7 DNA. Reaction mixtures were as described under Materials and Methods. 0.5 µg of T4 core enzyme and 0.37 µg of host sigma were used (●), or 0.5 µg of host core enzyme and 0.37 µg of host sigma (O).

and "open" complexes by Chamberlin[11]). Mangel and Chamberlin suggest that formation of the active preinitiation complex involves an opening of the DNA double helix since this formation is favored by low ionic strength and high temperatures. Active preinitiation complexes are measured by preincubating enzyme and DNA to allow their formation, and then determining RNA synthesis following addition of the four ribonucleoside triphosphates together with rifampicin.

To decide if the greater sensitivity of the T4 enzyme to KCl might be due to a greater inhibition by KCl of active preinitiation complex formation, these complexes were measured at different temperatures in the absence of salt, with 0.08 M KCl, and with 0.2 M KCl. The results are shown in Figure 3. Looking at the results with no salt addition, it can be seen that the T4 enzyme (A) requires higher temperatures for active preinitiation complex formation than the host enzyme (B). With salt, in the case of the T4 enzyme, 0.08 M KCl is quite inhibitory except at the two higher temperatures. At 43°C 0.08 M KCl stimulates complex formation. 0.2 M KCl almost completely inhibits complex formation. In the case of the host enzyme, 0.08 M KCl is inhibitory only at temperatures of 10°C and below. At temperatures above 20°C, it is stimulatory. 0.2 M KCl is less inhibitory than with the T4 enzyme. The results suggest that salt inhibits the T4 enzyme more because of its greater effect on

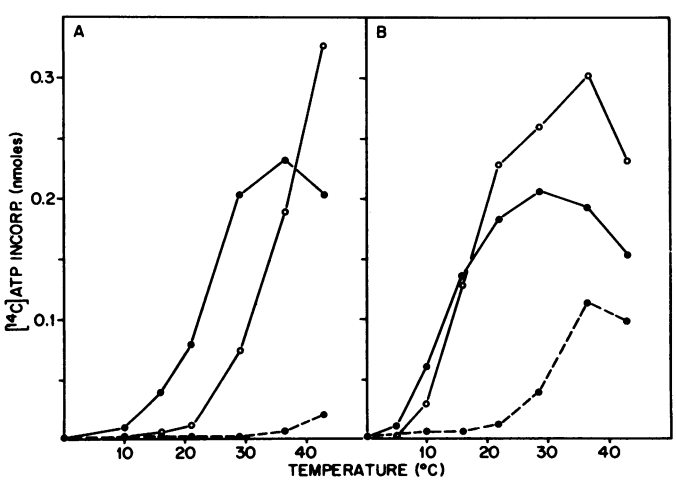


Figure 3. Effect of KCl and temperature on preinitiation complex formation with T4 enzyme (A) and host enzyme (B). Reaction mixtures and conditions were as described under Materials and Methods. In (A), 0.5 µg of T4 core enzyme and 0.45 µg of host sigma were used. In (B), 0.5 µg of host core enzyme and 0.45 µg of host sigma were used. No KCl (●—●); 0.08 M KCl (O—O); 0.2 M KCl (●---●).

active preinitiation complex formation.

Both the salt and temperature effects suggest that an initiation property of the T4 enzyme is altered. The alteration in initiation specificity may be of importance in the control of T4 transcription. What causes the altered specificity? T4 enzyme described here differs from host enzyme in containing an adenosine diphosphoribose substituent on the $\alpha$ subunits[12,13] and in its content of two polypeptides (one with a mol wt of 15,000, the other with a mol wt of 22,000[1]). The salt effect on T4 core enzyme is the same when it lacks the 22,000 mol wt polypeptide[1]. Either of the other two changes may be responsible for the altered salt sensitivity.

## ACKNOWLEDGEMENT

This investigation was supported by the Energy Research and Development Administration under contract with the Union Carbide Corporation.

## REFERENCES

1 Stevens, A. (1974) Biochemistry 13, 493-503
2 Stevens, A., and Rhoton, J. C. (1975) Biochemistry 14, 5074-5079
3 Stevens, A. (1976) in RNA Polymerase, pp. 617-627. Cold Spring Harbor Laboratory
4 Kleppe, R. K. (1975) FEBS Lett. 51, 237-241
5 Thomas, C. A., Jr., and Abelson, J. (1966) in Procedures in Nucleic Acid Research, pp. 553-561. Harper and Row, New York
6 Stevens, A. and Henry, J. (1964) J. Biol. Chem. 239, 196-203
7 Mueller, K. (1971) Molec. Gen. Genet. 111, 273-296
8 Matsukage, A. (1972) Molec. Gen. Genet. 118, 11-22
9 Mangel, W. F. and Chamberlin, M. J. (1974) J. Biol. Chem. 249, 3002-3006
10 Domingo, E., Escarmis, C. and Warner, R. C. (1975) J. Biol. Chem. 250, 2872-2877
11 Chamberlin, M. J. (1974) Ann. Rev. Biochem. 43, 721-775
12 Seifert, W., Rabussay, D. and Zillig, W. (1971) FEBS Lett. 16, 175-179
13 Goff, C. G. (1974) J. Biol. Chem. 249, 6181-6190