Volume 3 no.9 September 1976 Nucleic Acids Research

The kinetics of E.coli RNA polymerase

Adinah Solage and Howard Cedar

Department of Biochemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Received 17 March 1976

ABSTRACT

Using an assay specific for chain elongation of E. coli RNA polymerase the kinetics of this propagation reaction have been studied. The kinetic behaviour is consistent with the mathematical model formulated for this multi-substrate enzyme. The effect of increasing salt concentration on the kinetics of the reaction indicated that DNA unwinding is probably a necessary step in the propagation step, although this may not be the rate limiting step under all conditions.

INTRODUCTION

The synthesis of RNA on a DNA template has been studied extensively. It is commonly accepted that this process, catalyzed by RNA polymerase, takes place in at least three steps: initiation, chain propagation, and termination. Although many workers have attempted to elucidate the process of RNA chain initiation, very few have studied the mechanism and kinetics of chain elongation. Bremer (1), Hyman and Davidson (2) and Rhodes and Chamberlin (3) have derived the equations relevant to the kinetics of nucleoside triphosphate addition to a growing RNA chain and have presented experimental evidence supporting aspects of this model. Hyman and Davidson (2) have also shown that Actinomycin D inhibits RNA chain elongation specifically by interfering with the substrate addition of GTP and CTP.

Using an assay which measures the rate of RNA propagation per growing chain we have attempted to examine the mechanism of RNA chain elongation in vitro using E. coli RNA polymerase. In this paper we reconfirm the equations originally derived by Bremer (1) by showing that the addition of each nucleoside triphosphate is independent of the other three. In addition we have used high salt concentrations to strengthen the DNA hydrogen bonds

© Information Retrieval Limited 1 Falconberg Court London W1V 5FG England

and show that this causes a form of inhibition of RNA synthesis consistent with the idea that DNA unwinding is required for chain elongation.

MATERIALS AND METHODS

a) Materials – Nucleoside triphosphates were purchased from Calbiochem. [^{3}H] UTP (40 Ci/m mole) was obtained from New England Nuclear.

E. coli RNA polymerase was prepared by the procedure of Berg et al. (4) yielding a fraction V enzyme with a specific activity of 1100 units/mg, containing 0.8 molecules of σ component per molecule of RNA polymerase. One unit of activity as defined by these authors is equivalent to the incorporation of 1 nmole of ATP in 10 min.

Calf thymus DNA (GC = 41%) and chicken erythrocyte DNA (GC = 41%) were prepared as described previously (5). It had a weight-average size of 15,000 nucleotide pairs, and had about one single-strand break per strand. Only 3% of the DNA was digestible using a single strand specific nuclease. E. coli DNA (GC = 52%) was a gift of Dr. U. Bachrach and herpes virus DNA (GC = 68%) was obtained from Dr. Y. Becker.

b) Propagation assay – The rate of chain elongation per RNA molecule was measured by a modification of the procedure described by Cedar and Felsenfeld (5). In this method RNA polymerase is incubated with template at 37°C in 0.1 ml containing 10 mM Tris-HCl (pH 7.9), 1 mM $MnCl_2$ and 40 μM each of ATP, GTP and UTP. Under these limited conditions, RNA polymerase initiates synthesis but cannot elongate due to the lack of CTP. After 10 min this initiation reaction is complete (5). Chain elongation is then started by the addition of CTP in a volume of 0.5 ml. This mixture also contained [^{3}H] UTP (10 μCi), ATP, GTP, CTP and KCl as indicated. Propagation was determined by following the incorporation of radioactive UTP into TCA precipitable material after various time intervals up to 75 sec. Since TCA does not precipitate oligonucleotides containing less than 10 nucleotides (6), these polynucleotides, if present in the transcript, would not be detected by this assay system. In order to determine the propagation rate per RNA chain we have measured the number of RNA chains initiated in each reaction (5). It should be noted that in all cases, initiation was carried out under standard conditions, so that the number of initiated chains was always the same. In kinetic ex-

periments comparisons are always made between equal amounts of enzyme-DNA complex.

RESULTS

a) Rate of propagation – In all studies of enzyme kinetics it is essential to work under conditions where the initial reaction rate is linear during the period of measurement. In these studies we were interested in measuring the initial rate of RNA chain elongation independently of the initiation reaction. To this end we have chosen conditions which permit us to measure RNA chain elongation after initiation of all RNA molecules is completed. RNA polymerase was incubated for 10 min with DNA in the presence of three of the required four nucleoside triphosphates. This provides sufficient time for the initiation reaction to proceed to completion (5). Upon the addition of the fourth nucleoside triphosphate the chain elongation step begins. Propagation, when measured in this way was linear up to 75 sec. (Fig. 1). This rate is linear even when the nucleoside triphosphate concentration becomes rate limiting or when the amount of template is rate limiting. Using an assay which measures the number of initiated RNA molecules we determined that no new initiations occur during this short propagation period (5).

When propagation is measured under conditions where the nucleoside triphosphate concentrations were saturating (100 μM for each), we could calculate the chain elongation rate per RNA molecule. Using 2.3 μg of chicken DNA and 2.5 units of E. coli RNA polymerase we find an incorporation rate of 67 pmoles of UTP per min. Since 2.3 pmoles of RNA molecules are initiated under these conditions, this corresponds to a specific propagation rate of 100 nucleoside triphosphates per chain per min.

b) Kinetics of nucleoside triphosphate addition – The process of chain elongation is probably made up of several separate steps, including: A) nucleoside triphosphate addition and B) translocation of the polymerase from one site on the template to another. The kinetics of step A can be investigated by examining the rate of the reaction as a function of the nucleoside triphosphate concentrations. The equations describing this reaction have already been derived by Hyman and Davidson (2).

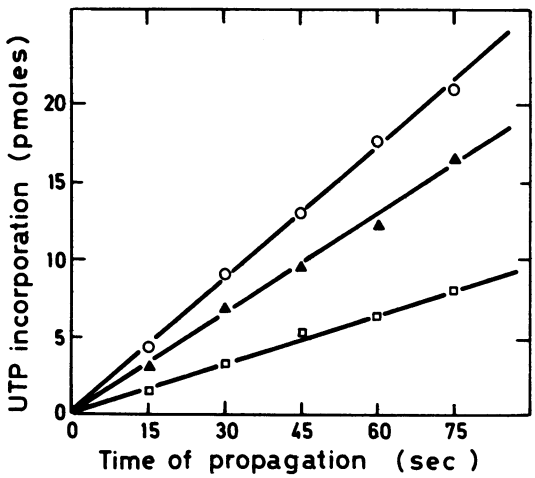


Figure 1. Chain elongation after initiation of RNA synthesis. Propagation was carried out as described (Materials and Methods) in a volume of 0.5 ml using 2.5 units of E. coli RNA polymerase and either 1.3 μg chicken DNA (O) (▲) or 0.5 μg chicken DNA (□). The concentrations of ATP, CTP and UTP were 40 μM and the concentration of GTP was either 40 μM (O) (□) or 6.7 μM (▲). Incorporation was determined from 100 μl aliquots at the indicated times and the results are expressed as pmoles UTP incorporated per 0.5 ml. Incorporation in the absence of DNA was 1.2 pmoles.

Let f_α be the mole fraction of nucleoside triphosphate α in the product RNA chain. Let t_α be the mean time for the addition of α to the chain, after the previous nucleoside triphosphate has been incorporated. Then the average velocity of incorporation, v, in units of nucleotides per unit time per chain, and the average time of incorporation per nucleotide (t), are given by

$$\frac{1}{v} = t = \sum_{\alpha = A,U,G,C} f_\alpha t_\alpha \qquad (1)$$

The quantities t_α are calculated as follows: Consider a polymerase-DNA-RNA complex, E_α, which is at a site where α is to be incorporated. The site is free, denoted by $E_\alpha{}^f$, or has the nucleoside triphosphate, denoted by S_α, bound in an enzyme-substrate complex, denoted by ES_α. We denote the

corresponding concentrations by $[E_\alpha]$, $[E_\alpha^f]$, $[S_\alpha]$, $[ES_\alpha]$.

The reactions for the reversible formation of the enzyme-substrate complex are (2)

$$E_\alpha^f + S_\alpha \underset{k_{2\alpha}}{\overset{k_{1\alpha}}{\rightleftharpoons}} ES_\alpha \tag{2}$$

and

$$[E_\alpha] = [E_\alpha^f] + [ES_\alpha] \tag{3}$$

The nucleoside triphosphate α becomes covalently attached to the RNA chain, and the enzyme moves on to the next site on the DNA in the reaction noted by

$$ES_\alpha \xrightarrow{k_{3\alpha}} P \tag{4}$$

The overall velocity of reactions (2) and (4) is obtained by the standard Michaelis Menten argument as

$$\frac{1}{[E_\alpha]} \cdot \frac{d[P]}{dt} = \frac{k_{3\alpha}[S_\alpha]}{K_\alpha + [S_\alpha]} \tag{5}$$

where

$$K_\alpha = \frac{k_{2\alpha} + k_{3\alpha}}{k_{1\alpha}} \tag{6}$$

Thus the velocity and propagation time for step α per chain are given by

$$\frac{1}{v_\alpha} = \frac{K_\alpha + [S_\alpha]}{k_{3\alpha}[S_\alpha]} = \frac{K_\alpha}{k_{3\alpha}[S_\alpha]} + \frac{1}{k_{3\alpha}} \tag{7}$$

Then, using equation (1), the overall rate of incorporation per chain is given by

$$\frac{1}{v} = t = \sum_{\alpha = A, C, G, U} \left[\frac{f_\alpha}{k_{3\alpha}} + \frac{f_\alpha K_\alpha}{k_{3\alpha}[S_\alpha]}\right] \tag{8}$$

In a typical experiment, one nucleoside triphosphate, S_β, is adjusted to a lower concentration than are the other three, and this concentration is

varied. Equation (8) predicts that a Lineweaver-Burke plot of $1/v$ versus $1/S_\beta$ should be a straight line with a slope of $f_\beta K_\beta / k_{3_\beta}$. The intercept on the $1/v$ axis at $(1/S_\beta) = 0$ is

$$\frac{1}{v}\,(1/S_\beta = 0) = \sum_{\alpha = A, G, C, U} \frac{f_\alpha}{k_{3_\alpha}} + \sum_{\alpha = \beta} \frac{f_\alpha K_\alpha}{k_{3_\alpha}[S_\alpha]} \qquad (9)$$

Bremer (1), Hyman and Davidson (2), and Rhodes and Chamberlin (3) have already presented data which support the rate law equation (8), by showing that Lineweaver-Burke plots of this type are indeed linear.

When the concentrations of three of the nucleoside triphosphates are held constant while the concentration of the fourth (β) is varied, one obtains a straight line when $\frac{1}{v}$ is plotted against $1/S_\beta$. The slope of this line ($f_\beta K_\beta / k_{3_\beta}$) should be independent of the concentrations of the other nucleoside triphosphates. As can be seen in Figures 2 and 3 the slope of such a graph indeed remains constant over a wide range of concentrations of the fixed nucleoside triphosphates.

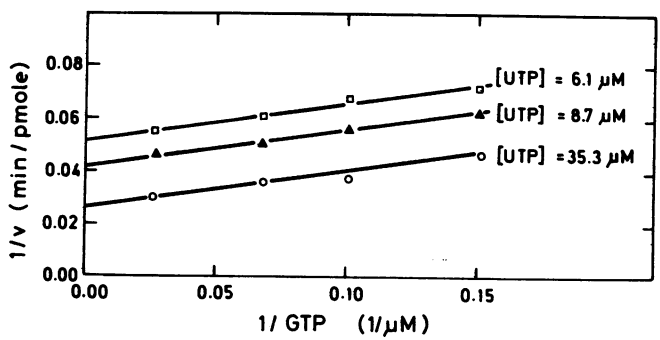


Figure 2. Dependence of propagation rate on GTP and UTP. Propagation was measured using 2.3 μg DNA and 2.5 units E. coli RNA polymerase. The concentrations of ATP and CTP were 40 μM.

c) Effect of T_m on chain propagation – Propagation can be thought to occur in at least three steps:

DNA unwinding ⟶ Translocation ⟶ Nucleotide addition

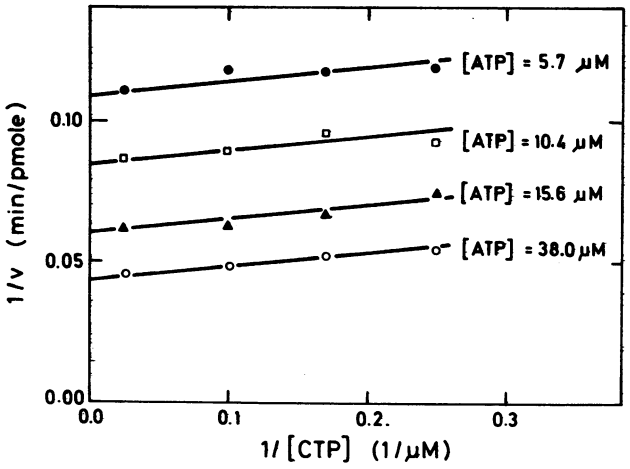


Figure 3. Dependence of propagation rate on CTP and ATP. Propagation was measured using 1.5 μg DNA and 2.5 units E. coli RNA polymerase. The concentrations of UTP and GTP were 40 μM.

The kinetics of nucleoside triphosphate addition have been investigated above and conform with the derived equations. Before the next nucleoside triphosphate is added to the growing chain, the enzyme must in some way move along the DNA to the next base pair (translocation). Although there is no conclusive proof that DNA must unwind in order for transcription to proceed, it has been suggested that this does occur (7). Using the propagation assay described in this paper we have attempted to clarify this point.

If DNA unwinding is indeed a necessary step in RNA synthesis then one should be able to inhibit transcription by making it more difficult to unwind the DNA. This can be done by carrying out propagation in increasing concentrations of salt, which is known to increase the melting temperature of the DNA (T_m) and thus inhibit unwinding.

As shown in Figure 4 propagation is indeed inhibited by increasing concentrations of KCl. In the case of chicken DNA, KCl concentrations of up to

0.4 M had no effect on the rate of chain elongation and above this there was a monotonic decrease in the rate. It should be noted that this is strictly an effect on propagation. In all of these experiments RNA synthesis was initiated in low salt; the KCl was only added after initiation was complete. Figure 4 shows a plot of 1/v versus 1/GTP at various salt concentrations. It is clear that parallel kinetics were obtained at all KCl concentrations up to 0.8 M.

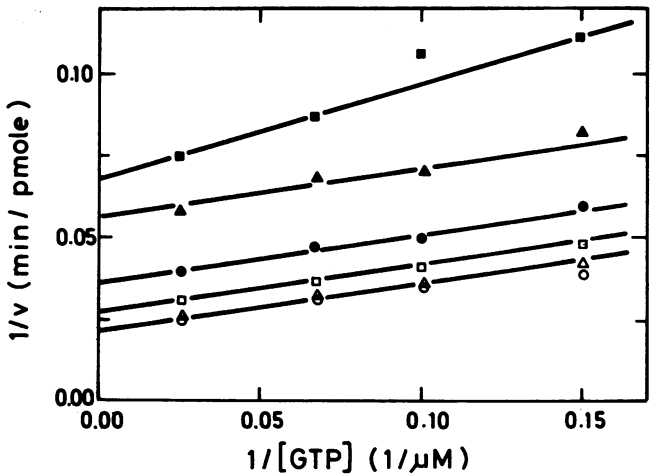


Figure 4. Effect of KCl on chain elongation. RNA synthesis was initiated using 2.5 μg DNA and 2.5 units *E. coli* RNA polymerase as described in Materials and Methods and propagation was started by the addition of 0.5 ml containing all of the nucleoside triphosphates and various concentrations of KCl. The final KCl concentrations for each set of experiments were as follows: no KCl (O), 0.4 M (Δ), 0.5 M (□), 0.6 M (●), 0.8 M (▲) and 1.0 M (■). The concentrations of ATP, CTP and UTP were 40 μM.

At 1 M KCl there is only a slight deviation from this pattern, which is referred to as uncompetitive inhibition (8) and is probably due to a change in the conformation of the enzyme itself. These kinetics are obtained in situations where the inhibitor affects a reaction step other than the one requiring the variable substrate. Since similar results were obtained for the other nucleoside triphosphates as well as GTP (unpublished results), we conclude that the effect of salt is not on nucleotide addition, but rather on one of the other steps, such as DNA unwinding or translocation.

It is well known that increasing salt concentrations effectively inhibit DNA unwinding. This effect has been quantitated by Schildkraut and Lifson (9) in the following equation:

$$T_m(°C) = GC/2.44 + 81.5 + 16.6 \log M \qquad (10)$$

where GC is the per cent GC content of the DNA and M is the salt concentration. If RNA synthesis were dependent on the local denaturation of the DNA one would expect the chain elongation rate to vary according to the T_m of the DNA under the propagation conditions.

If one assumes that the denaturation of DNA is a reversible reaction then one could write the following:

$$DNA_{native} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{DNA denatured} \qquad (11)$$

The effect of temperature on this reaction is expressed as

$$d \ln K = \frac{\Delta H}{RT^2} \qquad (12)$$

where $K = k_1/k_2$.

Integrating equation (11) from T_a to T_b one obtains

$$\ln \frac{K_b}{K_a} = \frac{\Delta H}{R} \left[\frac{1}{T_a} - \frac{1}{T_b} \right] \qquad (13)$$

In the case of DNA denaturation, when $T_b = T_m$, $K_b = 1$. Thus

$$\ln K_a = \frac{\Delta H}{R} \left[\frac{1}{T_a} - \frac{1}{T_m} \right] \qquad (14)$$

Now, if the rate of elongation indeed depends on DNA unwinding, it may be formulated as follows:

$$v \sim (v_{enzyme})(P) \qquad (15)$$

where v_{enzyme} is the inherent rate of migration of the RNA polymerase and P is the probability of finding the next segment of DNA unwound.

Thus:

$$K_a = \frac{P}{1 - P} \tag{16}$$

When $T_a \ll T_m$, as it is in these experiments then P is very small and is approximately equal to K_a, and v (the propagation rate) is then proportional to K_a. We may then conclude from equation (14) that

$$\ln (v) = \frac{C_1}{T_m} + C_2 \tag{17}$$

where C_1 and C_2 are constants.

If RNA synthesis depends on DNA unwinding a graph of ln(v) versus $1/T_m$ should be a straight line. Using the data in Figure 4 to obtain v and equation (10) to calculate the T_m for chicken DNA at the various salt concentrations, we find that RNA transcription depends on T_m as described by equation (17) (Fig. 5). Thus the effect of salt on transcription is related to its effect on the T_m of the DNA.

In order to confirm that this is an effect on the T_m of the DNA we have done this same experiment with DNA of different base composition. As shown in Figure 6 the inhibition was proportional to the T_m of the DNA regardless of the concentration of KCl. Thus at 0.4 M KCl there was no inhibition using chicken DNA (GC = 41%, T_m = 91.7°C) but considerable inhibition with E. coli DNA (GC = 52%, T_m = 96.2°C). Propagation on herpesvirus DNA (GC = 68%, T_m = 95.7°C) was inhibited even at a KCl concentration of 0.15 M whereas this concentration had no effect on either chicken DNA (T_m = 84.6°C) or E. coli DNA (T_m = 89.1°C).

d) Effect of base composition on kinetic parameters – Although high salt concentrations are capable of inhibiting transcription it is not clear whether DNA unwinding is the rate limiting step under conditions of low salt. We have measured the kinetic parameters of RNA polymerase chain elongation for two DNA templates with different GC content. We find that with either chicken DNA or herpesvirus DNA the enzyme has the same kinetic constants. Using the techniques described in Figures 2 and 3 we measured $K_\alpha/k_{3\alpha}$ for all four nucleoside triphosphates and find that these constants are

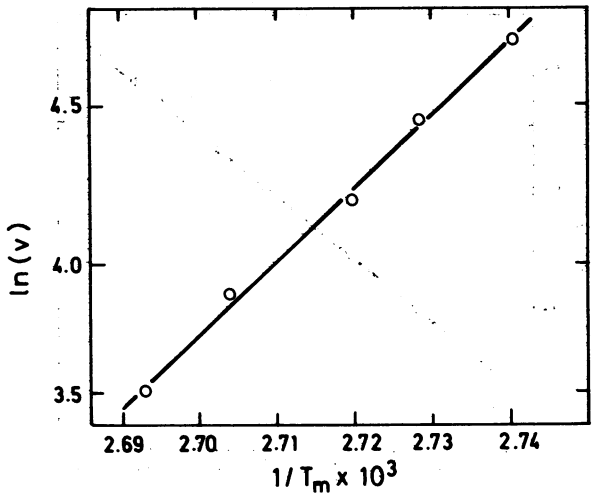


Figure 5. Relationship of T_m to propagation rate. The propagation rate (v) has been obtained by extrapolation of the data in Figure 4 to $1/S_\beta = 0$. v is expressed as the fraction of the maximal activity (obtained in the absence of KCl). T_m was obtained from the equation T_m (°K) = GC/2.44 + 354.5 + 16.6 log M. The GC content of chicken DNA is 41%. Although Schildkraut and Lifson (1965) state that this equation applies between 0.01-0.2 M, their data indicate that the equation is quite accurate even up to 1.0 M salt.

the same for both DNA samples (Table 1) despite the fact that under the conditions used in these experiments (10 mM Tris-HCl) chicken DNA has a T_m of 65°C whereas herpesvirus DNA has a T_m of 76°C. Thus within this range DNA unwinding does not seem to be the rate limiting step in transcription. At higher salt concentrations it is clear that DNA unwinding becomes rate limiting; judging from the data in Figures 5 and 6 this effect occurs when the effective T_m of the DNA is greater than 92°C.

DISCUSSION

RNA chain elongation has been studied using conditions which were designed to separate the propagation step from other steps in RNA synthesis. Under our conditions RNA synthesis is initiated in low salt in the presence of three nucleoside triphosphates producing RNA polymerase - DNA complexes

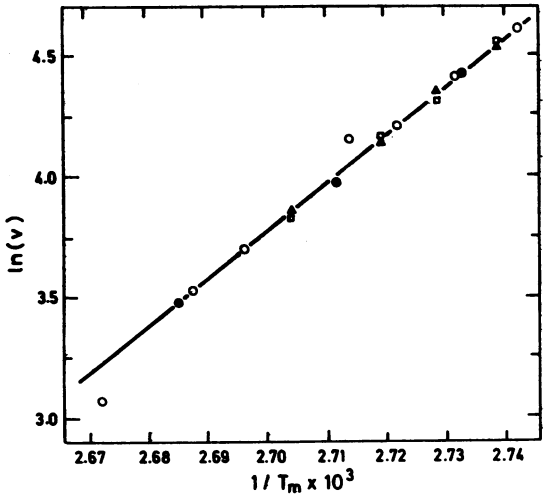


Figure 6. Relationship of T_m to propagation rate using DNA with different GC content. The propagation rate was measured using 2.5 units RNA polymerase, 40 μM ATP, CTP, GTP and UTP, 2.5 μg of either chicken DNA (Δ), calf thymus DNA (□), E. coli DNA (●) or herpesvirus DNA (O), and various KCl concentrations ranging from 0-0.8 M. The propagation rate is relative to the maximal rate obtained for each DNA (i. e., without added KCl). The maximum elongation rates for each DNA were as follows: chicken (39 pmoles UTP/min), calf thymus (35 pmoles UTP/min); E. coli (15.8 pmoles UTP/min) and herpesvirus (10.2 pmoles UTP/min). The T_m for each DNA and each KCl concentration was calculated from the equation found in the legend to Figure 5.

consisting of short polynucleotide chains which cannot be elongated without the fourth nucleoside triphosphate. Propagation is started by the addition of CTP. For a period of 2 min no new RNA chains are initiated, and chain elongation is linear with time (Fig. 1). One advantage of this system is that RNA propagation can be studied under various conditions (Figs. 2, 3, and 4) although initiation is always carried out under constant conditions.

Using this assay we have analyzed the kinetics of RNA propagation in vitro. Under conditions where three of the nucleoside triphosphates are held at a fixed concentration and the fourth (β) is varied, equation (8) predicts that the kinetic parameters for β are independent of the concentrations of α.

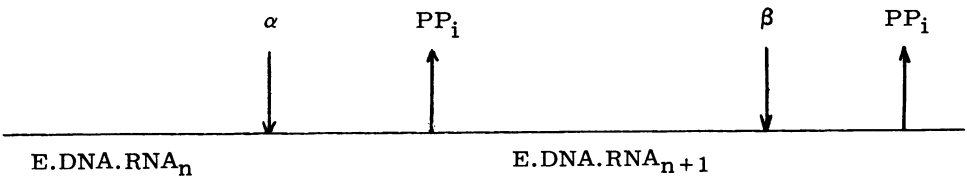
TABLE 1. Comparison of RNA polymerase activity on chicken and herpesvirus DNA

Nucleoside triphosphate	Chicken DNA		Herpesvirus DNA	
	slope	$k_\alpha/k_{3\alpha}$	slope	$k_\alpha/k_{3\alpha}$
	(μM) (nucleotides/min/chain)$^{-1}$			
GTP	0.074	0.35	0.112	0.33
UTP	0.102	0.35	0.062	0.39
CTP	0.016	0.08	0.034	0.10
ATP	0.176	0.61	0.085	0.53

Experiments as described in the legend to Figures 2 and 3 were performed for each nucleoside triphosphate using 2.5 units RNA polymerase and 1.8 μg DNA for each point. The slopes were obtained from plots of 1/v versus $1/S_\beta$, under conditions where the other three nucleoside triphosphates (α) were held constant at 40 μM. $K_\alpha/k_{3\alpha}$ was calculated from the slope using the relationship slope = $f_\alpha K_\alpha/k_{3\alpha}$. For chicken DNA $f_A = f_U = 0.29$ and $f_C = f_G = 0.34$. It should be noted that v (nucleotides/min/chain) was calculated from the incorporation of UTP after adjusting for the content of UTP in the RNA product.

Thus when 1/v is plotted against $1/S_\beta$, a straight line is obtained with slope $f_\beta K_\beta/k_{3\beta}$. We have demonstrated (Figs. 2 and 3) that this slope is indeed independent of the concentrations of the other nucleoside triphosphates. Similar results have been obtained by Rhodes and Chamberlin (3).

According to Cleland (8), a multisubstrate enzyme reaction can be visualized as follows:



where E.DNA.RNA_m is the enzyme-DNA complex with a growing RNA chain of length m and α and β nucleoside triphosphates. According to this scheme, 1/v versus $1/S_\alpha$ gives a straight line. If there are irreversible re-

action steps between the addition of α to the enzyme complex and the addition of β to the enzyme complex, then the slope does not depend on β. If there are no irreversible reaction steps then the slope does depend on the concentration of β. In the case of RNA polymerase, DNA unwinding, translocation, and release of PP_i are all irreversible reactions taking place between α and β. Thus our data (Figs. 2 and 3) are consistent with the proposed mechanism of RNA polymerase.

The question of whether local DNA denaturation is necessary for RNA synthesis has been investigated by several workers. Using theoretical considerations Florentiev and Ivanov (7) have suggested that RNA transcription could take place by Watson-Crick base pairing between the growing RNA chain and the DNA template if the DNA unwinds locally after changing from its usual B form to the less stable A form. In contrast to this Riley (10) claims that transcription could take place without DNA unwinding if the RNA chain grows in the wide groove of the DNA by a specific stereochemical interaction between the ribonucleoside triphosphates and the base pairs of the DNA. Kosaganov et al. (11) have used the kinetics of formaldehyde treatment to detect areas of local denaturation on native DNA. They find that during RNA synthesis, the amount of local unwinding increases. Saucier and Wang (12) have used nicked circular DNA to probe this problem. When this DNA is treated with compounds that cause local denaturation and then sealed by ligase, the amount of unwinding can be determined by the degree of superhelicity of the resulting sealed circular DNA. They found that the binding of RNA polymerase causes a small but definite unwinding of the DNA helix and that the magnitude of unwinding persists but does not increase during RNA synthesis. The most convincing evidence that DNA unwinding occurs during transcription has been presented by Bick et al. (13) who showed by electron microscopy that there are regions of DNA near RNA polymerase molecules which appear to be single stranded after treatment with formamide.

In this paper we have shown that RNA chain elongation is sensitive to the strength of the hydrogen bonds in the DNA double helix. Increasing the salt concentration and thus the T_m causes inhibition of RNA synthesis. This inhibition is not due to an effect on nucleoside triphosphate addition (Fig. 4), but is apparently due to its effect on the T_m of the DNA (Equation 15 and Fig. 5).

The fact that the inhibition is related to the T_m of the DNA was supported by experiments with DNA of differing GC content (Fig. 6). These data suggest that DNA unwinding is a necessary step in the synthesis of RNA. It should be noted that Bremer has observed an increase in the rate of RNA chain elongation at 0.2 M KCl using T4 DNA as template (14). We and other workers (15) have not been able to demonstrate this enhancement, which is probably dependent on the type of template used, the concentration of nucleoside triphosphates and the choice and concentration of divalent cation (14). All of our experiments have been done in the presence of 1 mM $MnCl_2$. Although the rate of chain elongation is different using Mg^{+2} (14), we would expect that the general form of salt dependence is similar for both divalent cations.

Although local DNA denaturation may be a necessary step, it may not be rate limiting under all conditions. Using 0.01 M salt, chicken DNA (GC = 41%) and herpesvirus DNA (GC = 68%) seem to provide the same rate of nucleoside triphosphate addition (Table 1). At higher salt concentrations the rate of DNA unwinding probably becomes rate limiting. We would like to suggest that this transition occurs when the DNA has an effective T_m of 92°C.

From our experiments it is difficult to predict what is the rate limiting step of RNA chain elongation *in vivo*. The rate of propagation measured *in vitro* in our system is far below the rate obtained *in vivo*. Using saturating concentrations of nucleoside triphosphates we obtained a maximal propagation rate of 100 nucleotides/min/chain. Manor *et al.* (16) have measured the rate of propagation in growing cultures of *E. coli* and find a rate of at least 46 nucleotides/sec/chain at 37.5°C.

In summary, we have shown that RNA synthesis *in vitro* conforms to the derived kinetic equation. In addition we have demonstrated that DNA unwinding is probably a necessary though not necessarily rate limiting step in the synthesis of RNA.

ACKNOWLEDGMENTS

We would like to thank Dr. Gary Felsenfeld and Dr. W. W. Cleland for their many helpful comments and suggestions. This project was supported by U. S. Public Health Service Grant No. GM20493.

REFERENCES

1. Bremer, H. (1967) Mol. Gen. Genetics 99, 362-371.
2. Hyman, R. W. and Davidson, W. (1970) J. Mol. Biol. 50, 421-438.
3. Rhodes, G. and Chamberlin, M. J. (1974) J. Biol. Chem. 249, 6675-6683.
4. Berg, D., Barrett, K. and Chamberlin, M. (1971) Methods in Enzymol. 21, 506-519.
5. Cedar, H. and Felsenfeld, G. (1973) J. Mol. Biol. 77, 237-254.
6. Cleaver, J. E. and Boyer, H. W. (1972) Biochim. Biophys. Acta 262, 116-124.
7. Florentiev, V. L. and Ivanov, V. I. (1970) Nature 228, 519-522.
8. Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 188-196.
9. Schildkraut, C. and Lifson, S. (1965) Biopolymers 3, 195-208.
10. Riley, P. A. (1970) Nature 228, 522-525.
11. Kosaganov, Yu. N., Zarudnaja, M. I., Lazurkin, Yu. S., Frank-Kamenetskii, M. D., Beabealashvilli, R. Sh. and Sovachkina, L. P. (1971) Nature New Biol. 231, 212-214.
12. Saucier, J. M. and Wang, J. C. (1972) Nature New Biol. 239, 167-170.
13. Bick, M. D., Lee, C. S. and Thomas, C. A. Jr. (1972) J. Mol. Biol. 71, 1-9.
14. Bremer, H. (1970) Cold Spring Harbour Symp. Quant. Biol. 35, 109-119.
15. Maitra, U. and Barash, F. (1969) Proc. Nat. Acad. Sci. U.S.A. 64, 779-786.
16. Manor, H., Goodman, D. and Stent, G. S. (1969) J. Mol. Biol. 39, 1-29.