I. ISOLATION OF TERNARY COMPLEXES AND THE KINETICS OF ELONGATION*

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SUMMARY

The chain elongation portion of enzymatic RNA synthesis has been studied by employing a ternary complex containing RNA polymerase, a DNA template, and product RNA. Ternary complexes were isolated by passage through a gel exclusion column and were shown to carry out RNA chain elongation under conditions that preclude RNA chain initiation or termination. The properties of the elongation reaction have been studied by employing ternary complexes formed with synthetic DNA templates. A simple ping-pong kinetic model was derived and was shown to fit the data obtained with alternating copolymer templates. The model is also able to account for the inhibition observed in high concentrations of nucleoside triphosphates. A general rate equation can be devised for RNA chain elongation with DNA templates of complex sequence if one assumes that the Michaelis constants for the substrates are independent of the DNA sequence. This assumption appears to hold for the various synthetic DNA templates that we have examined but has not been adequately tested for more complex templates.

Synthesis of RNA by DNA-dependent RNA polymerase in vitro proceeds in a series of steps. These normally include DNA template binding, RNA chain initiation, RNA chain elongation, and RNA chain termination and enzyme release (1). To understand the mechanism of RNA synthesis fully it is necessary to know the properties of each of these partial reactions. However, study of the partial reactions is not normally possible in the in vitro reaction system, where all of the steps occur together in a complex temporal sequence. Thus, to study the partial reactions, assay procedures must be devised which allow the characterization of each step separately from the others.

Studies of the chain elongation phase of RNA synthesis have shown that after RNA chain initiation a highly stable ternary complex containing RNA polymerase, template DNA, and the nascent RNA chain is formed. Chain elongation by this complex occurs without intermediate dissociation of any of the components from the complex (2-5). In order to minimize contributions due to initiation, chain elongation has been studied by employing complex trapped on filters (6), assaying in the presence of elevated salt (5, 7, 8), or using oligonucleotide primers (9, 10). Some kinetic analysis has also been done assuming that incorporation in a normal assay system reflects only the chain elongation phase of the reaction (11). However, recent studies make it clear that extensive RNA chain termination and re-initiation can occur in the complete reaction system (12-14); hence, study of the RNA chain elongation phase requires that these reactions be positively excluded. In addition, it is desirable to avoid the use of elevated salt concentrations to block RNA chain initiation, since such salt concentrations alter the rate of RNA chain elongation (15, 16) and also enhance RNA chain termination (12-14).

In this report we describe the isolation of ternary complexes of Escherichia coli RNA polymerase with several synthetic DNA templates and their complementary RNA products. These complexes actively carry out the chain elongation phase of RNA synthesis at a constant rate, even in the presence of the drug rifampicin, which selectively blocks RNA chain initiation. Hence the properties of the RNA chain elongation reaction can be studied with these complexes in the absence of complexities due to initiation or termination. A simple steady state model for enzymatic RNA chain elongation is presented.

EXPERIMENTAL PROCEDURE

Isolation of Ternary Complex—The following conditions were used to form the poly(d(A-T))-RNA polymerase-poly[r(A-U)] complex (called dAT complex). Three microliters of RNA polymerase (Fraction V (17); 600 DAT units, specific activity 12,000 units per mg) were added to a solution (0.5 ml) containing 0.05 M Tris-HCl (pH 8.0), 15 mM MgCl₂, 10 mM 2-mercaptoethanol, 11 mM poly[d(A-T)] nucleotide, and 1 mM UTP. This mixture was incubated for 1 min at 37°C and then ATP was added to give a final concentration of 1 mM. The reaction was incubated for 1 min and synthesis was stopped by the addition of 0.2 ml of 0.1 mM EDTA (pH 8.0). The mixture was chilled and washed on to a Bio-Gel P-100 column (2 X 80 cm) equilibrated with a solution containing 0.02 M Tris-HCl (pH 8.0), 1 mM MgCl₂, 0.1 mM diithiothreitol, 0.1 mM EDTA, and 5% glycerol; the complex was then eluted with the same buffer. The complex was located by ultraviolet absorption, and peak fractions were pooled and assayed for elongation activity. Procedures for the isolation of complexes with different templates were similar, except that the necessary nucleoside triphosphate substitutions were made. Complexes were stored at 4°C; the dAT complex was found to lose elongation activity slowly, with one-half of the activity being lost in approximately 1 week.

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Assay—Ternary complexes were assayed by employing a protocol similar to that used by Berg et al. (17), with the following modifications. Assay reaction mixtures (0.25 ml) contained 40 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 10 mM 2-mercaptoethanol, 50 mM KCl, and 10 μg of rifampicin per ml. After the reaction was terminated with ice-cold perchloric acid, 0.1 ml of yeast RNA (2 mg per ml) was added to each assay tube to facilitate precipitation of the labeled polynucleotide. One unit of elongation was defined as that amount of complex which incorporates one pmole of total nucleotide into polynucleotide in 10 min, all substrates at a concentration of 200 μM.

Error Analysis—Kinetic constants were calculated from the experimental data by the statistical procedures outlined by Cleland (18). This analysis also allows one to calculate variances in the fitted constants; these error estimates were in the range of 1 to 3% of the constants. Thus the experimental data obtained in any single experiment appear to fit quite closely the theoretical expressions used. The experimental deviation among Kₐ values obtained in 12 independent experiments with poly[d(A-T)] complexes showed a variance of from 10 to 12% (Table I), which lies in the expected range for the biochemical manipulations employed. It is assumed that the Kₐ determined for other templates varies to a similar extent (±10%).

Data on Nucleotides—Because of the very low Kₐ values for chain elongation, trace contamination of the unlabeled nucleoside triphosphate substrates employed in the reaction by other nucleoside triphosphates potentially can lead to serious errors in both Kₐ and Kₜ values and in interpretation of experiments. This is especially true in the determination of Kₜ values for nucleotide triphosphates. One can test the purity of the nucleoside triphosphate solutions by using an elongation complex with an alternating copolymer template and adding one substrate together with another nucleotide triphosphate which is not a substrate. Any incorporation reflects contamination with the missing substrate, and the concentration of the contaminant can be calculated from Equation 11 below. With this method it was found that the GTP present in our preparations was free of both ATP and UTP but contaminated with approximately 0.01% UTP. The GTP preparation employed was essentially free of both ATP and UTP. The trace contamination of the GTP was below that needed to affect any of the kinetic parameters qualitatively.

Materials—Escherichia coli polymerase was purified from E. coli B by the method of Berg et al. (17). The RNA polymerase used was Fraction V and was estimated to be 70% pure by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. It had a specific activity of 12,000 poly[d(A-T)] units per mg.

The synthetic DNA templates used were prepared from the deoxyribonucleoside triphosphates, utilizing E. coli DNA polymerase as cited in the following references: poly[d(A-T)] copolymer (19), poly[d(A-C)-d(T-G)] copolymer (20), poly[d(A)-d(T)] (21), and poly[d(A-G)-d(T-C)] (20). The products of the reaction were analyzed by polyacrylamide gel electrophoresis. The drug rifampicin efficiently blocks the initiation of new RNA chains (25) and has been routinely included in all chain elongation assays to preclude any contributions due to RNA chain initiation. There was no alteration in the rate of poly[d(A-T)] synthesis is directly proportional to the amount of ternary complex added over the assay range from 0.5 to 200 elongation units.

If one could determine the number of RNA polymerase molecules in active ternary complexes, one would be able to determine directly the rate of poly[r(A-U)] chain elongation and the concentration of active ternary complexes in a given solution. This has not yet been possible by any direct means that we have tried. An estimate of this number can be obtained by measuring the initial rate of poly[r(A-U)] synthesis in a normal RNA polymerase assay. The maximum specific activity reported for E. coli RNA polymerase holoenzyme is 24,000 units per mg (13, 17), which gives an initial rate of 34 s⁻¹, assuming that all enzyme molecules are active. At least 80% of the enzyme molecules are active in such a preparation, as measured by their ability to initiate T7 RNA chains (13, 24). Thus, unless there is an appreciable alteration of the rate of RNA chain elongation as the length of the poly[r(A-U)] chain is increased, a reasonable estimate of V_max for poly[r(A-U)] chain growth is probably 35 to 45 s⁻¹.

From this estimate one can approximate the concentration of active ternary complexes in the gel exclusion fractions and calculate the fractional recovery of active enzyme in ternary complexes as compared with the amount initially employed in the reaction (V_max in this case is calculated from the value of v observed in a standard elongation assay employing the equations determined below). These recoveries range from 5 to 7% of the active enzyme molecules initially added to the reaction in which ternary complex is formed. This low value may reflect an inactivation of enzyme in ternary complexes.

The kinetics of poly[r(A-U)] synthesis under these conditions are nonlinear [poly[r(A-U)] synthesis is directly proportional to the amount of ternary complex added over the assay range from 0.5 to 200 elongation units. The drug rifampicin efficiently blocks the initiation of new RNA chains (25) and has been routinely included in all chain elongation assays to preclude any contributions due to RNA chain initiation. There was no alteration in the rate of poly[d(A-T)] synthesis is directly proportional to the amount of ternary complex added over the assay range from 0.5 to 200 elongation units.

RESULTS

Chain Elongation by Poly[d(A-T)]-containing Complex

The proportion of ternary complexes obtained after gel exclusion chromatography were studied in greatest detail by employing the complex isolated with poly[d(A-T)] copolymer as template. Fractions eluted from the gel exclusion column which contained the poly[d(A-T)] copolymer template as monitored by absorbance at 250 nm were active in incorporating [α-3²P]AMP into polynucleotides in a standard chain elongation assay. The kinetics of poly[r(A-U)] synthesis under these conditions (Fig. 1) shows a constant rate of incorporation for at least 10 min, followed by a slow reduction in rate during the next 30 min. All rate measurements given in this paper were made in the initial, linear range of the elongation assay and the linearity of the initial rate was checked at several triphosphate concentrations whenever the assay conditions were varied. As expected, the rate of poly[r(A-U)] synthesis is directly proportional to the amount of ternary complex added over the assay range from 0.5 to 200 elongation units.

![Fig. 1](http://www.jbc.org/) Time course of poly[r(A-U)] chain elongation by poly[d(A-T)] ternary complexes. A standard elongation assay was performed, except that the concentration of ATP and UTP was 8 μM. The line represents the extrapolated initial velocity.
[r(A-U)] synthesis nor in the kinetic parameters obtained for poly[r(A-U)] synthesis ($V_{max}$, $K_a$, or $K_d$) when rifampicin was omitted from the reaction mixture or when the rifampicin concentration was increased to 500 µg per ml. These results indicate, first, that under standard elongation assay conditions there is essentially no poly[r(A-U)] chain termination and re-initiation by enzyme in active ternary complexes during the initial phase of chain growth. Second, it appears that there is no pool of active enzyme (in binary complexes, for example) which can initiate under these conditions. It also appears that rifampicin does not alter the kinetic properties of the enzyme in the ternary complex. This is expected, since Eilen and Krakow (26) have shown that rifampicin does not detectably bind to RNA polymerase in the ternary complex.

The effect of pH on the chain elongation assay is shown in Fig. 2. The rate of chain elongation shows a broad pH optimum, with a maximum at 8.0. This is similar to the relationship obtained for the over-all reaction (27-30). At pH 7.4 the rate of chain elongation is 65% of the optimal rate. Kinetic studies employing the procedures described below indicate that this decrease is due to an increase in the apparent $K_d$ value for ATP, whereas the $V_{max}$ for chain elongation is unchanged. A dependence of $K_d$ on pH (actually $\epsilon/K_d$) might be due to either an ionization of substrate or of a residue in the protein which alters or prevents substrate binding (31). The former possibility is excluded by the fact that the magnesium-nucleoside triphosphate substrate complexes have no pK in this range.

The dependence of the rate of a chain elongation on divalent cations (Fig. 3) shows that for manganese ion there is a sharp decrease at 0.2 to 0.3 M NH₄Cl. The rate of chain elongation is diminished at 1 M NH₄Cl; however, this reduction may not reflect a true decrease in the rate of elongation. At 1 M NH₄Cl there is rapid termination of poly[r(A-U)] chains growing in the ternary complex, and the rate measured in a 60-s assay may not be an accurate reflection of the true initial rate. Synthesis of RNA in the over-all reaction is completely eliminated by NH₄Cl concentrations of more than 0.3 M in the reaction mixture, probably due to the inhibition of DNA template binding by the salt (32, 33).

**Steady State Kinetic Model for Chain Elongation by Ternary Complexes**

A simple model for RNA chain elongation with a poly[d(A-T)] template postulates two states of the ternary complex. One state, which we will call $C_T$, contains an RNA chain with a 3'-terminal uridine residue and is a potential acceptor for an AMP residue. The complementary complex ($C_A$) contains a terminal adenosine residue. The simplest sequence of steps for chain elongation then involves:

$$C_T + MgATP \rightleftharpoons C_T(MgATP)$$

1. G. Rhodes and M. Chamberlin, unpublished observations.
The kinetic equation (34), this treatment gives two linear relationships which previously had been held constant.4 As expected from Equation 5 (34), this treatment gives two linear relationships from which the constants are obtained.

It is also predicted from Equation 5 that $K_S$ values for MgATP and MgUTP in the elongation reaction, the intercepts on the ordinate of each of the lines in Fig. 5 were graphed in a secondary plot (Fig. 6) as a function of the reciprocal of the concentration of the nucleotide which previously had been held constant.4 As expected from Equation 5 (34), this treatment gives two linear relationships from which the constants are obtained.

It is also concluded that $K_S$ values for MgATP and MgUTP can be obtained directly, by varying one substrate while the other is present in great excess. However, this procedure cannot be used without additional information because high concentrations of a competing nucleoside triphosphate competitively inhibit chain elongation (see below).

By applying the steady state kinetic treatment derived above, we have determined several $K_S$ values for the alternating copolymers poly[d(A-T)], poly[d(A-BU)], poly[d(A-C)-d(T-G)], and the homopolymer pair poly[d(A)]-d(T). In the case of the poly[d(A-T)] template several analogues of UTP have also been tested. Although the number of different templates and substrates for which kinetic parameters have been determined is not large, several conclusions appear from the data, which is tabulated in Table I.

First, within experimental error, the values of $K_A$ and $K_U$ do not vary for the different templates (Note that the value of $K_A$ measured with the alternating copolymer templates has to be multiplied by 2 in order to be compared with the value obtained from poly[d(A)-d(T)].) This is discussed further under “Discussion”.

By using the binding constant for ATP to Mg$^+$ measured by O’Sullivan and Perrin (42), one can show that the triphosphates are completely saturated with Mg$^+$ in these experiments.

**Fig. 5.** Kinetic analysis of RNA polymerase chain elongation by poly[d(A-T)] ternary complexes. a, poly[d(A-T)] ternary complex (60 elongation units) was incubated with varying concentrations of ATP while the UTP concentration was held constant. Initial velocities were measured by following the incorporation of [3H]-UMP and the reciprocal of this quantity was plotted as a function of the reciprocal of the ATP concentration. The concentrations of UTP were: $\bullet$--$\bullet$, 1.0 $\mu$M; $\square$--$\square$, 2.0 $\mu$M; $\Delta$--$\Delta$, 8.0 $\mu$M; and $\bigcirc$--$\bigcirc$, 200 $\mu$M. b, the concentration of ATP was held constant and that of UTP was varied. Initial velocities were measured by following the incorporation of [3P]-AMP. The results were plotted as in a. The ATP concentrations were: $\bullet$--$\bullet$, 1.06 $\mu$M; $\square$--$\square$, 9.12 $\mu$M; $\bigtriangleup$--$\bigtriangleup$, 3.18 $\mu$M; $\bigcirc$--$\bigcirc$, 6.35 $\mu$M.

**Fig. 6.** Secondary plots of the data in Fig. 5. The 1/v intercepts of the curves in Fig. 5 were plotted as a function of the reciprocals of the concentrations of the fixed substrate for each curve. The intercepts on the abscissae are the reciprocals of the kinetic constants; in this case $K_A$ is 8.3 $\mu$M and $K_U$ is 18 $\mu$M. The varied quantities are: $\bigtriangleup$--$\bigtriangleup$, ATP; $\Delta$--$\Delta$, UTP; $\bigcirc$--$\bigcirc$, ATP.
centrations of nucleoside triphosphates within the bacterial cell are 10 to 1000 times higher than the $K_s$ values (36, 37).

The $K_s$ values for several nucleotide analogues were also measured in order to determine how structural perturbations might affect the reactivity. All of these $K_s$ values are much higher than those found with the natural substrates, and the maximal velocity of the reaction is also affected in some instances. The alteration of the $V_{max}$ makes quantitative interpretation of the analogue results difficult (see below).

**Inhibition of Chain Elongation by Nucleoside Triphosphates and Derivatives**

**Inhibition by Substrate Nucleoside Triphosphates**—The derivation of Rate Equation 3 assumed that only the nucleoside triphosphate which is to be incorporated into an RNA chain would bind to the complex (Reactions 1 and 3). Since misincorporation by RNA polymerase is very low (38), one might expect that either (a) a complex $CT$ is unable to bind UTP at all or (b) $CT$ binds UTP but the complex is unable to carry out phosphodiester bond formation. In the latter case, if we raise the UTP concentration high enough one might expect to see inhibition of poly[r(A-U)] chain elongation by UTP even though it is a substrate. As shown in Fig. 7, such inhibition is observed.

It is a simple matter to extend the model for elongation to account for inhibition of this sort. For example, if one is concerned with elongation by a dAT complex, then one can consider that the complex called $CT$ defined as the complex which must insert ATP as the next nucleotide, can instead bind MgUTP to form a complex $CT(MgUTP)$ which can react no further:

$$CT + MgUTP \rightarrow CT(MgUTP)$$  \hspace{1cm} (6)

Following the method of Cleland (40), one can show that the addition of Reaction 6 to Reactions 1 to 4 adds a single term to the rate equation yielding

$$V/V = 1 + K_u/U + (K_d/A)(1 + U/K'_u)$$  \hspace{1cm} (7)

where $K'_u$ is an inhibition constant for the inhibition of MgATP incorporation by MgUTP. The constant $K'_u$ in Equation 7 is the equilibrium constant for the binding of a noncomplementary nucleoside triphosphate to the ternary complex and is not simply a collection of rate constants, as in the case of $K_u$ values.

The numerical value for $K'_u$, which is about 15 $\mu$M, is much less than $K'_d$, which is of the order of magnitude of 1 mM. This means that at MgUTP concentrations with which one must be concerned with substrate inhibition the ratio $K'_d/U$ is very much less than 1 and can be neglected. In this case Equation 7 can be simplified to

$$V/V = 1 + (K_d/A)(1 + U/K'_u)$$  \hspace{1cm} (8)

Thus at elevated MgUTP concentrations, MgUTP can be neglected as a substrate, and one can proceed to determine $K'_d$ as though MgUTP were simply a competitive inhibitor. Experimentally, if one chooses a UTP concentration and determines $v$ while varying the ATP concentration, one obtains a linear double reciprocal plot as before (Fig. 7). At a second UTP concentration, a new linear relationship is obtained in which the slope is changed but not the $1/v$ intercept. This behavior is characteristic of competitive inhibition in which the substrate and inhibitor compete for the same form of the enzyme (40, 41).

By an appropriate replot of the data shown in Fig. 7 one can determine numerical values for the $K_d$. A summary of the results is given in Table II.

**Inhibition by Triphosphates That Are Not Substrates**—A substance which is not a substrate for the elongation reaction can also react with the complex in a competitive manner. In this case it can potentially interact with both forms of the complex:

$$CT + MgATP \rightarrow CT(MgATP)$$

$$CT + MgUTP \rightarrow CT(MgUTP)$$

and introduces two new factors into the rate equation

$$V/V = 1 + (K'_d/A)(1 + 1/K'_d) + (K'_u/U)(1 + 1/K'_u)$$  \hspace{1cm} (11)

where $K'_d$ and $K'_u$ are not necessarily equal. One can measure these constants by selecting the proper experimental conditions. For example, to measure $K'_d$, one can work at substrate concentrations in which the UTP concentration is much greater than

**Table I**

**Summary of $K_s$ values**

Reaction conditions are described in Figs. 5 and 6. Error estimates are expressed as standard deviations of 12 independent estimates of both $K_A$ and $K_U$ with a poly[d(A-T)] template. As discussed under "Experimental Procedure" the error in the $K_s$ values obtained with other templates is at least 10%. The maximal velocities using the substrates FUTP and dUTP are 0.46 and 0.36 that of UTP.

| $K_s$ | Template          | $\mu$M |
|-------|-------------------|--------|
| $K_A$ | 7 ± 0.8           | 11.3   |
| $K_U$ | 10 ± 3            | 5      |
| $K_BU$| 94                | 18     |
| $K_DU$| 50                | 15     |
| $K_AU$| 90                |        |

![Fig. 7. Inhibition of poly[r(A-U)] chain elongation by elevated concentrations of UTP. Standard elongation reactions with poly[d(A-T)] ternary complexes (60 elongation units) were used. The ATP concentration was varied and initial rates were measured at various fixed UTP concentrations. The concentrations of UTP used in these experiments were: O---O, 0.50 mM; Δ---Δ, 5.0 mM; U---U, 10.0 mM.](http://www.jbc.org/Downloadedfrom)
FIG. 8. Kinetic analysis of inhibition by GTP of chain elongation by the poly[d(A-T)] ternary complex. For each experiment a fixed concentration of GTP was employed and the concentration of one substrate was varied while that of the second was held constant. In a the UTP concentration was 200 μm and the concentrations of GTP were: ○, 0.0 mm; △, 2.5 mm; □—□, 4.0 mm; ●—●, 7.0 mm. In b the ATP concentration was fixed at 100 μm and the GTP concentrations were: △—△, 0.35 mm; □—□, 3.16 mm; ○—○, 9.45 mm.

\[ K_U \] while the concentration of ATP is of the order of magnitude of \( K_A \). One can neglect the term containing \( (K_U/U) (1 + I/K_{AU}) \), and the equation simplifies to the form of Equation 8. An example of this type of inhibition is shown in Fig. 8, where high concentrations of GTP are used to inhibit the activity of the poly[d(A-T)] complex.

\( K_A \) values for various templates are shown in Table II. We find that within experimental error \( K_A = K_A^* \) and \( K_A^* = K_D^* \). This indicates that MgCTP and MgGTP can interact equally well with both CA1 and CT. There are considerable differences in both the size of the bases and the positions of the reactive groups between the substrates MgATP and MgUTP, used by these complexes, and the inhibitors MgCTP and MgGTP. The results suggest that this binding site is not appreciably altered in the experimentally determined quantities may be as much as 20%.

| Template       | Inhibitor | Inhibition of A incorporation | Inhibition of U incorporation |
|----------------|-----------|-------------------------------|-------------------------------|
| Poly[d(A-T)]   | ATP       | 1.8                           | 3.2                           |
|                | UTP       |                               |                               |
|                | CTP       | 3.8                           | 3.9                           |
|                | GTP       | 2.0                           | 3.8                           |
|                | ADP       | 0.6                           |                               |
|                | UDP       | 3.2                           |                               |
|                | Tripolyphosphate | 1.3                         |                               |
| Poly[d(A-C)·d(T-G)] | UTP | 1.3                          |                               |
|                | GTP       | 2.3                           |                               |
|                | CTP       | 2.9                           |                               |

Our experiments show that ternary complexes containing RNA polymerase, template DNA, and a growing RNA chain can be isolated free from substrates under conditions such that a portion of the enzyme molecules is still able to elongate RNA chains actively. The initial rate of this elongation reaction is constant for a reasonable length of time, and this result, together with the insensitivity of the reaction to the presence of the drug rifampicin, assures that the repeated initiation of RNA chains or chain termination events do not occur to a significant extent in this system under standard conditions. Consequently these ternary complexes provide an attractive approach to the study of the kinetic and biochemical properties of the chain elongation phase of enzymatic transcription.

The rate of RNA chain elongation carried out by these ternary complexes follows simple steady state rate equations for the several synthetic DNA templates that we have tested. For DNA templates of alternating nucleotide sequence, a so-called “ping-pong” kinetic model is obtained in which the enzyme oscillates alternately between two states, each of which is specific for a different substrate (34).

The \( K_A \) values obtained in our equations are each composed of collections of individual microscopic rate constants. However, \( K_A \) values cannot be compared directly unless they are composed of analogous rate constants. To be more specific, for the series of DNAs poly[d(A-T)], poly[d(A-C)·d(T-G)], poly[d(A-G)·d(C-T)], and poly [d(A)·d(T)], the respective \( K_A \) values are directly comparable in the first three cases because they contain analogous rate constants (Reactions 1 to 4). The \( K_A \) values obtained for the homopolymer pair, poly [d(A)·d(T)], is not directly comparable since the constants \( V \) and \( K_A \) in its rate equation, \( v/V = K_A/A \), do not contain the same rate constants as those in Equation 5, the rate equation for a template of repeated dinucleotide sequence.

To overcome this difficulty, one can consider poly[d(A)·d(T)] not as a repeating homopolymer but rather as the repeating copolymer poly[d(A-A)·d(T-T)]. By employing the approach we have discussed above, this model generates the rate equation \( v/V = 1 + K_A/A + K'A/A \), where \( K_A \) and \( K'A \) are the \( K_S \) values for adding the first and second AMP in the sequence. By virtue of the symmetry of this template, these quantities must be equal and the rate equation for this template becomes \( v/V = 1 + 2K_A/A \). Hence, if \( K_A\text{h} \) is the measured \( K_A \) value on the homopolymer template and \( K_A\text{d} \) is the \( K_A \) value that one would obtain by considering the template as a repeating dinucleotide sequence, then \( K_A\text{h} = 2K_A\text{d} \).

In order to compare the \( K_A \) values obtained with various synthetic repeating templates, we can define an “equivalent homopolymer value,” \( K_{A,h} \). The \( K_A \) values for dinucleotide templates must then be doubled to generate the equivalent \( K_{S,h} \) values. These expressions can easily be generalized to obtain an equivalent homopolymer value for any repeating nucleotide sequence; in this case \( K_{S,h} = K_{S,exp}/f_S \) is the measured \( K_S \) value for that template and \( f_S \) is the fraction of nucleotide \( S \) in the RNA product. Some values of \( K_{S,h} \) obtained in this paper and from the literature are tabulated in Table III.

The preceding analyses can be proven more formally by deriving the expression for \( K_S \) in terms of the microscopic rate constants. In this way one can show that if unique \( K_{S,h} \) values are to exist then all rate constants following binding of the substrate must be independent of the nucleotide incorporated; that is, once the substrate is bound, the rate of any isomeriza-
Table III
Equivalent homopolymer $K_S$ values

The equivalent homopolymer $K_S$ values were calculated as described under "Discussion." If no information was available as to the base frequencies of the RNA synthesized, this was approximated by using base frequencies of the DNA template (47). Abbreviations used in the template column are: CT, calf thymus; ML, Micrococcus lysodeikticus; ST, Salmonella typhimurium. The parameters $K_S$ and $f_S$ are defined in the text.

| $K_A$ | $K_U$ | $K_C$ | $K_G$ | $f_A$ | $f_U$ | $f_C$ | $f_G$ | Template | Initiation Bypassed | Unvaried Concentration | Ref |
|-------|-------|-------|-------|-------|-------|-------|-------|---------|----------------------|------------------------|-----|
| 14    | 32    | -     | -     | .5    | .5    | -     | -     | poly[d(A-T)] | yes                  | 200                    | this paper |
| 10    | 36    | -     | -     | .5    | -     | .5    | -     | poly[d(A-C) .d(T-G)] | yes                  | 200                    | " "   |
| 11.3  | -     | -     | -     | 1.0   | -     | -     | -     | poly[d(A-T)] | yes                  | 200                    | " "   |
| 18    | -     | -     | -     | .5    | .5    | -     | -     | poly[d(A-BU)] | no                    | 400                    | 10    |
| 50    | 50    | -     | -     | .5    | .5    | -     | -     | poly[d(A-T)] | yes                  | 400                    | 6     |
| 26    | -     | 60    | -     | .5    | .5    | -     | -     | poly[d(A-T)] | yes                  | 400                    | 6     |
| 53    | -     | 100   | -     | .285  | .285  | .215  | .215  | CT       | no                    | 100A, C                | 20    |
| 114   | 93    | 75    | 42    | .14   | .14   | .36   | .36   | poly[d(A-T)] | no                    | 300G                   | 11    |
|       | 36    | -     | -     | .5    | .5    | .5    | .5    | poly[d(A-C) .d(T-G)] | no                    | " "   |
|       | 30    | -     | -     | .5    | -     | .5    | -     | poly[d(A-C) .d(T-G)] | no                    | " "   |
|       | 12    | -     | -     | .5    | .5    | .5    | -     | poly[d(A-T)] | no                    | " "   |
|       | 135   | -     | -     | 1.0   | -     | -     | -     | poly[d(A)] | no                    | " "   |
| 130   | 102   | 279   | 233   | .285  | .285  | .215  | .215  | CT       | no                    | 800                    | 48    |
|       | -     | -     | 20    | -     | -     | 1.0   | -     | poly[rC] | no                    | " "   |
| 21    | 33    | 46    | 46    | .24   | .24   | .26   | .26   | ST       | no                    | 250                    | 30    |
| 28    | 21    | 28    | 42    | .285  | .285  | .215  | .215  | CT       | no                    | 800                    | 27    |
|       | -     | 26    | -     | -     | -     | .36   | -     | T4       | no                    | 200                    | 11    |
| 60    | 1338  | 19    | 46    | .26   | .26   | .24   | .24   | T7       | yes                   | 30 .80                  | 7     |
| 89    | 414   | 86    | 277   | .28   | .28   | .22   | .22   | CT       | yes                   | 94                     | 8     |

One can extend the kinetic treatment to include any template by assuming that the $K_S$ values do not depend on the DNA sequence. In this case the equivalent homopolymer value, $K_{S,h}$, becomes an intrinsic $K_S$ value for the substrate, and one can write a general rate equation

$$V/v - 1 + \sum f_S K_{S,h}$$  \hspace{1cm} (12)

where the sum is over all nucleotides contained in the RNA product. Both Bremer (11) and Hyman and Davidson (9) devised a kinetic treatment of RNA chain elongation based on these assumptions. The equation they derive is identical with Equation 12.

If $K_S$ does depend on a nucleotide sequence, the kinetics of chain elongation will still fit an equation of the form $V/v = 1 + \sum S K_S/\Sigma$ but the values of $K_S$ will depend on the template DNA used in the measurements. Our extremely limited data indicate that $K_{A,A}$ does not vary appreciably for the templates poly[d(A-T)], poly[d(A) .d(T)], and poly[d(A-C) .d(T-G)]. However, the $K_{A,A}$ values estimated from the literature for DNA templates are quite different in some instances, and the possible dependence of $K_{S,h}$ values on nucleotide sequence needs to be studied more thoroughly.

Empirically, the values of $K_S$ for ATP and UTP do not vary when poly[d(A-BU)] replaces poly[d(A-T)] as template, despite the fact that the former forms a considerably more stable helical structure. This suggests that denaturation or opening of the DNA strands is not rate-limiting in the elongation phase of RNA synthesis. The values of $K_S$ do vary appreciably when base analogues such as FUTP, BUTP, and deoxyuridine triphosphate replace UTP; variation of apparent $K_S$ values in the complete RNA polymerase reaction had been reported previously for these analogues (30). The interpretation of these variations is made difficult by the lack of information in our experiments as to the true value of $K_S$ for base-stacking or base-pairing interactions. Instead, all of the analogues have sub-stantially higher $K_S$ values than does UTP and this suggests that interactions between the protein and the substrate rather than interactions between substrate and the DNA template are most critical in determining $K_S$. Again, this fits our tentative conclusion that $K_S$ does not show a substantial dependence on nucleotide sequence for these DNA templates.

The elongation of RNA chains by ternary complexes is inhibited at elevated concentrations of nucleoside triphosphates. This effect has been reported previously with the complete RNA polymerase reaction as an inability of noncomplementary nucleoside triphosphates to inhibit synthesis of polynucleotides when templates of restricted base composition were employed (43). This "low efficiency inhibition" was attributed to a low general affinity of RNA polymerase in the ternary complex for nucleoside triphosphates (43) and is consistent with a model of RNA polymerase which postulates a single common site on the enzyme...
at which nucleoside triphosphates are bound for RNA chain elongation (38, 43).

Our results confirm the earlier reports of low efficiency inhibition and show that this inhibition reflects a general ability of nucleoside triphosphates and related compounds to inhibit RNA chain elongation competitively. Alternative possibilities to explain this inhibition, such as complexing of metal ions in the reaction at elevated nucleoside triphosphate concentrations (39), can be ruled out. Since the values of \( K_s \) obtained for different nucleoside triphosphates reflect the affinity of the enzyme in the ternary complex for that nucleoside triphosphate, a comparison of \( K_s \) for the different forms of the ternary complex allows a rough probe of the structure of the complex. One can ask, does the ternary complex \( C_4 \) have the same affinity for GTP as does \( C_T \)? The results indicate that, within the limits of accuracy, all forms of ternary complex have equal affinity for noncomplementary nucleoside triphosphates. This result has two important negative consequences; it makes less likely models of RNA polymerase in the ternary complex which postulate (a) four separate sites, one for each nucleoside triphosphate, or (b) a single site which is altered appreciably in its conformation by the DNA template base that it is to read. Instead, the data fit well with a model proposed some time ago (38, 43) in which selection of the correct nucleoside triphosphate proceeds through a random, weak binding of all possible substrates at a common site on the enzyme followed by a conformational change to form a much stronger complex when the correct triphosphate enters that site. Studies of the specificity of incorporation of base analogues into RNA by RNA polymerase make it very likely that transition into this second “active” complex is determined primarily by the ability of the base of the incoming triphosphate to fit into a site on the enzyme that also contains the template base to be read (44). It would be of interest to probe the conformation of this complex with complementary nucleoside triphosphate analogues which are unable to form phosphodiester bonds (45, 46); the isolated ternary complexes that we have described and the availability of a steady state kinetic model for elongation by these complexes provide an attractive system for such a study.

A large number of studies of the kinetics of RNA synthesis by RNA polymerase in vitro and in vivo have been carried out since the initial reports of the enzyme. A comparison of the equivalent homopolymer values for phage and bacterial DNAs may be somewhat higher than the values obtained for synthetic templates. However, a definite conclusion regarding this possibility must await careful measurements in which initiation is bypassed and the unvaried substrate is judiciously chosen, as discussed above.

Several groups have shown that the identity of a base analogue can profoundly influence the nearest neighbor frequencies with which it is incorporated into an RNA chain when synthesis occurs in the presence of both analogue and the naturally occurring nucleoside triphosphate. For example, when a mixture of UTP and CTP are used for RNA synthesis, CTP preferentially follows A or U, whereas UTP preferentially follows G or T. If these differences reflect differences in incorporation in the elongation phase of the reaction, then, since there appears to be no alteration of \( K_s \) with different nearest neighbors, how can these nearest neighbor preferences be expressed? The simplest answer would be that the maximal rate of incorporation, \( V \), can vary appreciably with the nucleoside sequence. Again, it would be extremely valuable to have a method for measuring this parameter in the current system. The notion that the rate of RNA chain elongation is sensitive to the nucleotide sequence of the region being transcribed has been suggested by others (1, 44, 46), and a number of models can be imagined in which this phenomenon could play an important role in the process of RNA synthesis on DNA templates and its regulation.

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