Ribonucleic Acid Polymerase from *Micrococcus luteus (Micrococcus lysodeikticus)*. IV. Effect of Rifampicin and Oligomers on the Homopolymer-Directed Reaction*

Patricia A. Straat and Paul O. P. Ts'o

ABSTRACT: The inhibitory action of the antibiotic rifampicin on DNA-dependent RNA synthesis and on poly U dependent poly A synthesis in the *Micrococcus luteus* RNA polymerase system has been examined. Its effect is similar to that reported for DNA-dependent RNA synthesis by *Escherichia coli* RNA polymerase which suggests that rifampicin is a specific inhibitor of initiation also for the *M. luteus* enzyme. Further studies regarding initiation have confirmed that oligonucleotides of A stimulate poly U directed poly A synthesis. For optimal stimulation, an oligo A concentration stoichiometric with the template concentration is required, suggesting that the mechanism of this stimulation may involve complex formation between poly U and the oligomers. However, although nucleoside triphosphates protect RNA polymerase against the inhibitory action of rifampicin in DNA-directed RNA synthesis, $A(pA)_4$ does not protect RNA polymerase against the inhibitory action of rifampicin in poly U directed poly A synthesis.

Recent research on the mechanism of the RNA polymerase synthetic reaction has emphasized the initiation phase of the reaction which can be separated from the subsequent phase, chain elongation. Thus, it has been demonstrated for *Escherichia coli* RNA polymerase that most RNA product chains are initiated with purine nucleoside triphosphates (Bremer *et al.*, 1965; Maitra and Hurwitz, 1965) and that the binding of the initial nucleoside triphosphate to the DNA–RNA polymerase complex results in a stabilization of that initiation complex (Anthony *et al.*, 1966; Khesin *et al.*, 1967; di Mauro *et al.*, 1969). That a specific protein component is involved in this initiation step is becoming apparent (Travers and Burgess, 1969). Other recent studies on initiation concern the antibiotic rifamycin which specifically inhibits initiation but not elongation. Rifamycin binds to *E. coli* RNA polymerase (Wehrli *et al.*, 1968b; di Mauro *et al.*, 1969), causes complete inhibition before the onset of synthesis, but has no effect after the reaction has begun (Sippel and Hartmann, 1968). Rifamycin may exert its effect by preventing the binding of the first nucleoside triphosphate to *E. coli* RNA polymerase as suggested by di Mauro *et al.* (1969). It does not, however, prevent the binding of the enzyme to its template (Umezawa *et al.*, 1968). Other studies have shown that rifamycin is without effect on the RNA polymerase systems isolated from bacteria resistant to the antibiotic (Wehrli *et al.*, 1968a,b; Tocchini-Valentini *et al.*, 1968; Ezekiel and Hutchins, 1968). While the significance of these deviations is yet unknown, it is known that resistant enzymes also fail to bind rifamycin (Wehrli *et al.*, 1968b).

For RNA polymerase systems with homopolynucleotide templates, initiation has been studied by the introduction of oligonucleotides complementary to the template into the reaction mixture. Thus, using *E. coli* RNA polymerase and single-stranded poly A[1] or poly U templates, Niyogi and Stevens (1965) demonstrated that oligonucleotides complementary to the template enhance incorporation rates as well as reduce the lag period observed at low substrate concentration. Since a free 3′-OH was necessary for stimulation and since some oligomers were incorporated into the initial portion of the product, these oligomers were considered to be product initiators. The oligomers presumably bypass the rate-limiting initiation step of the synthetic reaction. The stimulatory effect of pentaadenylate and pentauridylate on the synthesis of poly A and poly U, respectively, with single-stranded poly U or poly A as template has also been demonstrated for the RNA polymerase system from *Micrococcus luteus*, formerly known as *Micrococcus lysodeikticus*.[2] More recently, Niyogi and Wilton (1969) have shown a correlation between the optimal temperature of the oligonucleotide stimulation and the melting temperature of the oligonucleotide–template complex.

In continuing our studies on *M. luteus* RNA polymerase and homopolynucleotide templates (Straat *et al.*, 1968, 1969; Straat and Ts'o, 1969), we have examined the initiation phase of these reactions and have confirmed the stimulatory action of $A(pA)_4$ and $A(pA)_6$ on the poly U directed synthesis of poly A. Further studies have shown that rifampicin, a derivative of rifamycin, is a specific inhibitor of initiation with *M. luteus* RNA polymerase for both DNA-directed synthesis of RNA and poly U directed synthesis of poly A. However, when rifampicin is added to the oligomer-stimulated reaction,

* From the Department of Radiological Sciences, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205. *Received June 20, 1969.* This study was supported by grants from the National Science Foundation (GB 5483) and from the National Institutes of Health (GM-16066-01).

[1] All the abbreviations follow the Revised Tentative Rules (1965) of the IUPAC–IUB Combined Commission on Biochemical Nomenclature (*Biochemistry 5*, 1445, 1966).

[2] According to the catalog of the American Type Culture Collection (1968), *Micrococcus lysodeikticus* is now reclassified as *Micrococcus luteus* (ATCC 4698).

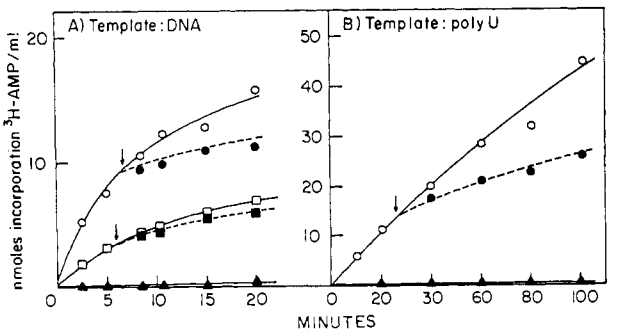

FIGURE 1: Effect of rifampicin on *M. luteus* RNA polymerase. (A) Incorporation of [^{3}H]AMP under standard assay conditions with calf thymus DNA as template is shown for 200 (□) and 800 nmoles (○) of *each* of GTP, CTP, UTP, and [^{3}H]ATP. The effect of rifampicin addition to each reaction mixture approximately 7 min after its onset is represented by solid symbols and dashed lines. The effect of rifampicin addition before the onset of synthesis at both substrate concentrations is also shown (△). (B) Incorporation of [^{3}H]AMP under standard assay conditions with poly U as template and 1200 nmoles of [^{3}H]ATP as substrate is represented by open circles. The effect of rifampicin addition after 25 min is represented by ●. Incorporation in the presence of rifampicin which was added before the onset of synthesis is represented by ▲.

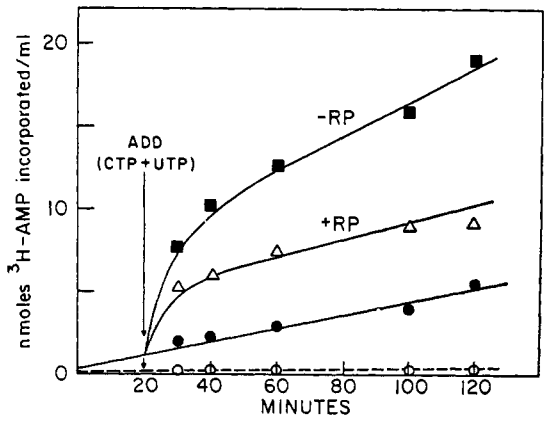

FIGURE 2: Protection against rifampicin inhibition by preliminary incubation of the RNA polymerase–DNA complex with purine nucleoside triphosphates. RNA polymerase was preincubated with DNA, manganese, ATP, and GTP (—, ●). At the indicated time, a mixture of CTP and UTP was added to one portion of this reaction mixture (■). To a second portion, 10^{-5} M rifampicin (RP) was added just prior to the addition of CTP and UTP (△). RNA polymerase was also preincubated with DNA, manganese, rifampicin, ATP, and GTP (-----, ○). At the indicated time, CTP and UTP were also added to this mixture.

nucleotide incorporation is inhibited. Preliminary incubation of RNA polymerase, poly U, and A(pA)$_4$ fail to prevent the inhibitory effect of rifampicin.

Methods and Materials

Highly purified RNA polymerase from *Micrococcus luteus* was prepared as previously described (Straat *et al.*, 1968) by modifications of the procedure of Nakamoto *et al.* (1964). The enzyme was stored in precipitated form in 50% saturated ammonium sulfate and 0.2 M phosphate buffer (pH 7.5) at −70° where it was stable indefinitely. Prior to use, the enzyme was thawed and diluted to an appropriate concentration before addition into the reaction mixture.

Calf thymus DNA was purchased from Sigma Chemical Corp. whereas poly U, poly A, and the dephosphorylated oligomers, A(pA)$_4$ and A(pA)$_6$, were purchased from Miles Laboratories. Poly U and poly A had sedimentation coefficients of 6 and 9 S, respectively. Solutions of poly U, poly A, and the oligomers were adjusted to an appropriate concentration (moles of polynucleotide phosphorus) using molar extinction coefficients of 9.2×10^3, 10.5×10^3, and 11.0×10^3 at 260 mμ, respectively (Straat *et al.*, 1968; Cantor and Chin, 1968). Melting curves of polymer complexes were performed with a Cary Model 15 equipped with a heating jacket and a thermistor probe. Nucleoside triphosphates were purchased from Schwarz BioResearch. Solutions of [^{3}H]ATP were lyophilized before adjusting the specific activity to 8.3 μCi/μmole. The rifamycin derivative used was rifampicin which was purchased from Mann Laboratories.

RNA polymerase activity with DNA as template was measured essentially as described by Nakamoto *et al.* (1964). Per milliliter, each standard reaction mixture contained 100 μmoles of Tris buffer (pH 8.0), 2.5 μmoles of $MnCl_2$, 1.6 μmoles of spermidine hydrochloride, 200 μg of calf thymus DNA, and 200–800 nmoles each of CTP, GTP, UTP, and [^{3}H]ATP. The reaction was conducted at 30°. The assay with poly U as template has also been previously described (Straat *et al.*, 1968). Per milliliter, this reaction mixture consisted of 100 μmoles of Tris buffer (pH 7.5), 2.5 μmoles of $MnCl_2$, 100 nmoles of poly U, and 400–1200 nmoles of [^{3}H]ATP, as indicated. Addition of either A(pA)$_4$ or A(pA)$_6$ consisted of 50 nmoles of polynucleotide phosphorus unless otherwise indicated. Rifampicin was added in excess at a concentration of 10 nmoles/ml. Unless otherwise indicated, all homopolymer reactions were conducted at 25°.

Each reaction was started with the addition of 10–40 μg of protein after a 15-min preliminary incubation of the other ingredients at the reaction temperature. At various times throughout the synthetic reaction, 0.1-ml aliquots were streaked onto 2 × 3 cm filter pads of Whatman No. 3 paper. Each pad was quickly placed into a beaker containing 1 l. of a solution of 5% trichloroacetic acid and 1% sodium pyrophosphate. When all aliquots had been collected, they were washed batchwise as previously described (Straat and Ts'o, 1969), dried under an infrared lamp, and counted to 2% accuracy in a Beckman Model 200B liquid scintillation counter using a toluene–2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene counting solution (Straat *et al.*, 1968).

Results

Effect of Rifampicin. Rifampicin has been examined for its effect on the DNA-dependent synthesis of RNA catalyzed by *M. luteus* RNA polymerase. As shown in Figure 1A, 10^{-5} M rifampicin completely inhibits the DNA-directed reaction when the antibiotic is added before the onset of [^{3}H]AMP incorporation. This result is identical with that found with the *E. coli* enzyme (Sippel and Hartmann, 1968) and demonstrates the sensitivity of the *M. luteus* enzyme to the antibiotic. Addition of rifampicin to the reaction after

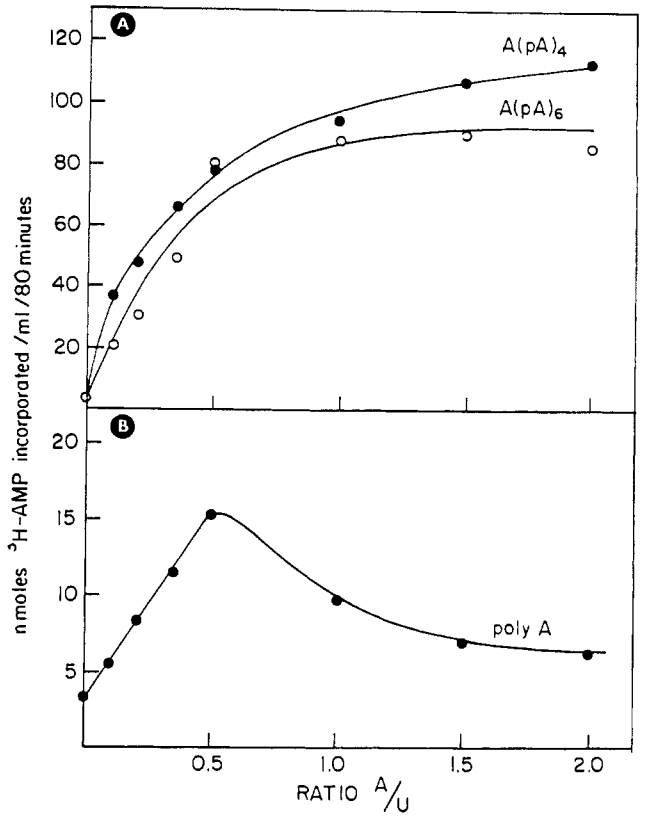

FIGURE 3: Effect of concentration. (A) Of the oligonucleotides A $(pA)_4$ (●) and $A(pA)_6$ (○) on poly U directed [^{3}H]AMP incorporation as expressed in terms of an A:U ratio. (B) Of poly A on poly U directed [^{3}H]AMP incorporation as expressed in terms of an A:U ratio. Each standard 1.0-ml reaction mixture contained 100 nmoles of poly U, 400 nmoles of [^{3}H]ATP, and 0–200 nmoles of oligo A or poly A to give the indicated A:U ratio. The temperature of the reaction was 25°.

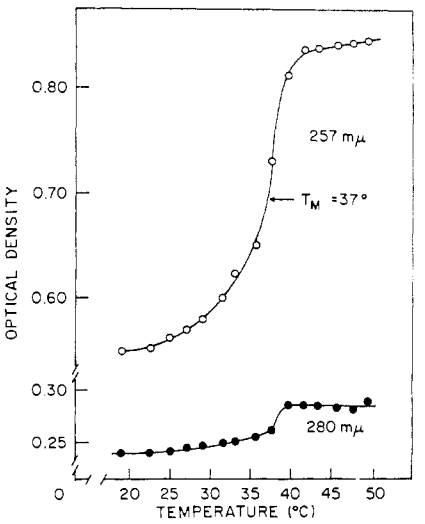

FIGURE 4: Melting temperature of the $A(pA)_4$·poly U complex. The melting mixture contained 0.1 M Tris (pH 7.5), 1.5×10^{-3} M $MnCl_2$, 50 nmoles/ml of poly U, and 25 nmoles/ml of $A(pA)_4$. The change in optical density as a function of temperature is shown for 257 mμ (○) and for 280 mμ (●).

synthesis has begun, however, shows some inhibition at high substrate concentration but little or no inhibition at low substrate concentration. Thus after the onset of synthesis, rifampicin is not as potent an inhibitor of the reaction, a result again similar to that found with the *E. coli* enzyme (Sippel and Hartmann, 1968). This differential effect of rifampicin before and after the onset of synthesis suggests that rifampicin is probably a specific inhibitor of initiation for the *M. luteus* RNA polymerase as well as for the *E. coli* enzyme. The small inhibition following late rifampicin addition observed at high substrate concentration may demonstrate some continued initiation throughout the reaction.

The recent experiments of di Mauro *et al.* (1969) have shown that preincubation of *E. coli* RNA polymerase, T_4 DNA, and mixtures of three nucleoside triphosphates (lacking either CTP or UTP) protects RNA synthesis against the action of rifampicin. Thus, following the addition of rifampicin and the missing nucleoside triphosphates significant incorporation was observed. We have performed similar experiments with *M. luteus* RNA polymerase. As seen in Figure 2, in the presence of *M. luteus* RNA polymerase, calf thymus DNA, ATP, and GTP, a small amount of [^{3}H]-AMP incorporation is observed. Addition of CTP and UTP to this mixture results in considerable RNA synthesis. If rifampicin is added prior to the addition of ATP and GTP to the enzyme–template complex, [^{3}H]AMP incorporation is completely inhibited. Later addition of CTP and UTP does not over come this inhibition. However, if rifampicin is added after ATP and GTP, but before CTP and UTP, RNA synthesis does occur. Although rifampicin is somewhat inhibitory under these conditions, preincubation with ATP and GTP did afford protection against the action of rifampicin. These results are qualitatively similar to those of di Mauro *et al.* (1969) who suggest that " . . . rifampicin presumably competitively inhibits the binding of the first nucleotide to the RNA polymerase, perhaps by binding to the same sites."

The action of rifampicin on poly U directed poly A synthesis (Figure 1B) is similar to its action on DNA-directed RNA synthesis. At a substrate concentration of 12×10^{-4} M ATP, a concentration known to be optimal (Straat and Ts'o, 1969), a differential effect of the antibiotic is again observed. Thus, 10^{-5} M rifampicin completely inhibits synthesis when added before the onset of the reaction. This is true even if the enzyme is added to a reaction mixture containing both rifampicin and ATP. Although separate experiments established this concentration as at least a tenfold excess for total inhibition before synthesis, 10^{-5} M rifampicin only partially inhibited (about 55%) when added 25 min after the onset of synthesis. This late inhibition again indicates continued initiation during the reaction. It should be noted, however, that while the early inhibition always occurs, the extent of the late inhibition seems to depend upon the ATP concentration (see Figure 6). These results are currently under investigation in our laboratory. The finding that rifampicin inhibits initiation by *M. luteus* RNA polymerase for the homopolymer reactions as well as for the DNA-directed reaction is expected since rifampicin is known to bind the enzyme rather than the template (Wehrli *et al.*, 1968b; Umazawa *et al.*, 1968).

Effect of Oligomers. The effect of dephosphorylated

TABLE I[a]

Poly U	nmoles of Poly A	$A(pA)_4$	[^{3}H]AMP Incorp (nmoles/80 min)
100			2.4
100	50		8.7
100	100		5.8
100		50	82.8
100	50	50	61.5
100	100	50	33.2

[a] Influence of poly A and $A(pA)_4$ on poly U directed incorporation of [^{3}H]AMP. Each standard reaction mixture, conducted at 25°, contained 400 nmoles of [^{3}H]ATP and the indicated amount of each polynucleotide. Incorporation for each reaction was essentially linear over the 80-min time period.

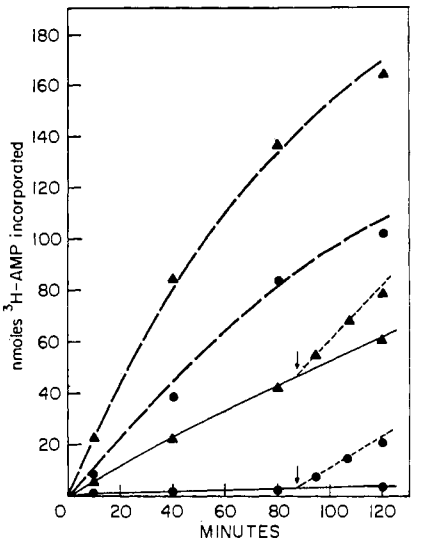

FIGURE 5: Poly U directed [^{3}H]AMP incorporation in the presence (---) and absence (—) of $A(pA)_4$ as a function of time. The standard 1.0-ml reaction mixture contained either 400 (●) or 1200 nmoles (▲) of [^{3}H]ATP. $A(pA)_4$ was added either before the onset of the reaction (——) or 87 min (see arrow) after the onset of each reaction (-----).

adenylate oligomers on the poly U directed reaction has been studied. Both $A(pA)_4$ and $A(pA)_6$ dramatically stimulate poly A synthesis as directed by poly U with *M. luteus* RNA polymerase (Figure 3A). The stimulation is concentration dependent, a fact also noted for the *E. coli* RNA polymerase system (Niyogi and Stevens, 1965) although it has not been extensively studied. In our system, the optimal oligomer concentration is similar for both $A(pA)_4$ and $A(pA)_6$ and is somewhat greater than half that of the template concentration. This relationship shows a requirement for stoichiometric, rather than catalytic, amounts of oligo A and suggests that complex formation between oligo A and the poly U template may be an integral part of the mechanism of stimulation by oligomers. Such a complex apparently does exist in our assay since, under simulated assay conditions (0.1 M Tris, pH 7.5, 1.5×10^{-3} M $MnCl_2$), the melting curve of the complex showed a T_m of 37° (Figure 4). At 25°, the temperature of the reaction mixture, the complex remained intact. Niyogi and Wilton (1969) have also implicated the importance of complex formation by showing a correlation between the optimal temperature of oligomer stimulation and the T_m of the oligomer–polymer complex. With *E. coli* RNA polymerase, poly U and the oligomers $A(pA)_{3-5}$, the optimal temperature of stimulation was within 1–2° of the melting temperature of the complex. We have confirmed this correlation in our system since with *M. luteus* RNA polymerase, poly U, and $A(pA)_4$, the optimal temperature of stimulation is about 34°, 3° below the T_m.

Complexes of poly U and oligomers of A are known to assume a triple-stranded structure (Cantor and Chin, 1968). The oligomer effect, however, is probably not due exclusively to the formation of a triple-stranded complex. Thus, for directing AMP incorporation, the optimal ratio for templates consisting of poly A·poly U complexes corresponds to the triple-stranded complex, poly A·2 poly U (Fox *et al.*, 1964; Figure 3B; Table I). However, this latter complex is approximately ten times less effective than the triple-stranded complex between oligo A and poly U in directing poly A synthesis (Table I).

The effect of $A(pA)_4$ on [^{3}H]AMP incorporation as directed by single-stranded poly U, double-stranded (poly A· poly U), and triple-stranded (poly A·2poly U) is compared in Table I. For all three templates, $A(pA)_4$ is stimulatory. While poly A does inhibit the $A(pA)_4$ stimulation of poly U directed incorporation, $A(pA)_4$ nonetheless does produce a six- to sevenfold stimulation of the reactions directed by the double- and the triple-stranded templates. Since a complex between $A(pA)_4$ and double-stranded poly A·poly U has never been found (Michelson *et al.*, 1967; Felsenfeld and Miles, 1967), this observation, in contrast to the above results, suggests that complex formation may not be necessary for oligomer stimulation.

The oligomer effect has been further studied by examining the kinetics of poly U directed AMP incorporation when $A(pA)_4$ is added both before and after the onset of the synthetic reaction (Figure 5). At both ATP concentrations examined, the reaction kinetics are essentially linear. When $A(pA)_4$ is added before the onset of the reaction, the stimulation is large at the lower ATP concentration and relatively small at the higher ATP concentration. These results are similar to those of Steck *et al.* (1968). Figure 5 also shows that if $A(pA)_4$ is not added until 85 min after the beginning of the reaction, it is still stimulatory. Furthermore, at both ATP levels, the extent of this stimulation is within 30% of that seen when $A(pA)_4$ is added at the onset of the reaction. These experiments with oligomers again suggest that initiation may occur throughout the reaction and agree with the results of Figure 1B in which 40–70% of the late incorporation is resistant to rifampicin inhibition. Thus, the results confirm the conclusion that the late rifampicin inhibition observed in Figure 1 does reflect continued initiation.

Effect of Rifampicin on the Oligomer-Stimulated Reaction. In our experiments with calf thymus DNA, preincubation of RNA polymerase, DNA, and two nucleoside triphosphates protected subsequent RNA synthesis against rifampicin

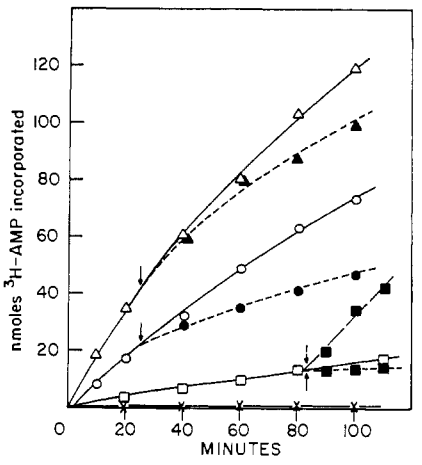

FIGURE 6: Effect of rifampicin on the $A(pA)_4$ stimulation of poly U directed [^{3}H]AMP incorporation. The standard reaction mixture with $A(pA)_4$ (50 nmoles) present at the onset of synthesis contained 100 nmoles of poly U, and either 400 (○) or 1200 nmoles (△) of [^{3}H]ATP. Rifampicin was also added to each of these mixtures 25 min after the onset of synthesis (see arrows). The incorporation following rifampicin addition is represented by ● and ▲. Rifampicin has also been added to each of these mixtures before the onset of synthesis; this incorporation is also shown (×—×). In the absence of $A(pA)_4$ at the onset of the reaction with 1200 nmoles of [^{3}H]ATP, incorporation of [^{3}H]AMP is shown by □. At 83 min (see arrow), $A(pA)_4$ was added to this reaction mixture either with (-----) or without (——) the simultaneous addition of rifampicin. The resulting incorporation is represented by ●.

TABLE II[a]

Contents of Preliminary Incubation	Reaction Started with	[^{3}H]AMP Incorp (nmoles/ 80 min)
RNA polymerase, poly U, $A(pA)_4$, $MnCl_2$	[^{3}H]ATP	47.8
RNA polymerase, poly U, $A(pA)_4$, $MnCl_2$, rifampicin	[^{3}H]ATP	0.4
RNA polymerase, poly U, $A(pA)_4$, $MnCl_2$	Rifampicin, then [^{3}H]ATP	0.2

[a] Effect of preliminary incubation of RNA polymerase, poly U, and $A(pA)_4$ on rifampicin inhibition of poly U directed [^{3}H]AMP incorporation. Each 1.0-ml reaction mixture contained 0.1 M Tris (pH 7.5), 2.5×10^{-3} M $MnCl_2$, 12×10^{-4} M [^{3}H]ATP, 100 nmoles of poly U, 80 nmoles of $A(pA)_4$ and, where present, 10^{-5} M rifampicin. The contents of each reaction mixture were incubated for 30 min at 25° before the addition of [^{3}H]ATP to start the reaction.

inhibition (Figure 2). Since a similar experiment could not be conducted with RNA polymerase, poly U, and ATP without product synthesis, we have examined the protective effect of $A(pA)_4$ against the inhibitory action of rifampicin on poly U directed [^{3}H]AMP incorporation. The results presented in Figure 6 show that $A(pA)_4$ has no influence on the inhibitory action of rifampicin. In the presence of $A(pA)_4$, rifampicin added at the onset of synthesis completely inhibits the reaction at both high and low ATP concentrations. Preliminary incubation of RNA polymerase, poly U, $A(pA)_4$, and manganese for 30 min before the addition of rifampicin and ATP still does not protect the system against rifampicin inhibition (Table II). Furthermore, rifampicin is also somewhat inhibitory 25 min after the onset of the $A(pA)_4$-stimulated synthesis, although, again, the extent of inhibition differed at the two ATP concentrations examined. Thus, preliminary incubation of the enzyme with $A(pA)_5$ either before or during the synthetic reaction does not protect against rifampicin inhibition. It is of considerable interest that whereas the initiating nucleotides, ATP, and GTP, can protect against rifampicin inhibition, initiating oligomers can neither furnish this same protection against rifampicin nor override rifampicin inhibition.

Discussion

The results presented above indicate that the mechanism whereby oligomers of A stimulate poly U directed AMP incorporation may involve complex formation between the oligomers and poly U. Similar conclusions have been reached by Niyogi and Stevens (1965) and by Niyogi and Wilton (1969). On the other hand, oligomers of A also stimulate AMP incorporation directed by double- and triple-stranded templates of poly A and poly U. Complex formation between oligomers and these templates probably does not occur. Other examples of oligomer stimulation can also be cited where the oligomer–polymer complex either is quite unstable or is not known to exist. The oligomers ApA and $A(pA)_2$, for example, form complexes with poly U that melt 17 and 8°, respectively, lower than their optimal temperature of stimulation (Niyogi and Wilton, 1969). Oligo U and poly A also do not form stable complexes (Michelson, 1968; Niyogi and Wilton, 1969) although oligo U stimulates poly A directed UMP incorporation (Niyogi and Stevens, 1965; Steck *et al.*, 1968; Niyogi and Wilton, 1969). Further, the optimal temperature of this stimulation is about the same as for the corresponding oligo A–poly U complexes (Niyogi and Wilton, 1969). To harmonize these observations, Niyogi and Wilton (1969) proposed that " . . . the RNA polymerase may considerably stabilize complex formation between oligouridylates and poly A." It is interesting that this "stabilization" effect is large for the poly $A \cdot U(pU)_n$ system, small for the poly $U \cdot A(pA)_{1-2}$ system, and nil for the poly $U \cdot A(pA)_{3-5}$ system. Such an "enzyme stabilization" concept requires the formation of an enzyme–oligomer–template complex. Since $A(pA)_4$ stimulates the reaction directed by double-stranded poly A · poly U (Table I), this would require the formation of an enzyme–$A(pA)_4$–(poly A · poly U) complex. It should be noted, however, that a complex between oligo A and (poly A · poly U) is not known to exist (Michelson *et al.*, 1967; Felsenfeld and Miles, 1967).

The formation of the initiation complex in the DNA-directed reaction involves the complexing of the initiating nucleoside triphosphate with the DNA–enzyme complex. The existence of this complex was first demonstrated by Anthony *et al.* (1966) who showed that purine nucleoside

triphosphates effectively protected the DNA–enzyme complex against dissociation by high salt. Similar results have been obtained by di Mauro *et al.* (1969) who, in addition, showed that rifampicin prevented purine stabilization of the DNA–enzyme complex to high salt dissociation if added before ATP and GTP but not if added after ATP and GTP. These results are qualitatively similar to the protective effect of triphosphates against rifampicin inhibition of RNA synthesis (di Mauro *et al.*, 1969; Figure 2). These authors therefore suggested that rifampicin may inhibit the binding of the first initiating nucleotide to RNA polymerase. The work of Khesin *et al.* (1967) and of Novak and Doty (1968) suggest an explanation for the protective effect of nucleoside triphosphates against rifampicin. In the experiments of Khesin *et al.*, nucleoside triphosphates added to *E. coli* RNA polymerase and T2 DNA stabilized the enzyme–DNA complex to high salt. Maximum stabilization was achieved by the presence of all four triphosphates. No single nucleoside triphosphate could achieve this stabilization with the exception of ATP which could achieve 30–80% of the total stabilization. Similarly, the nucleoside triphosphates also increased the stability of RNA polymerase, bound in an enzyme–template complex, to the proteolytic action of trypsin. Such protection suggests that a conformational change of RNA polymerase occurs upon the binding of the nucleoside triphosphates to the enzyme–template complex. Novak and Doty (1968) also have reported increased resistance of RNA polymerase to proteolytic enzymes during transcription. These conformational changes could account for the protection afforded by nucleoside triphosphates against rifampicin inhibition. Thus, either rifampicin or ATP could elicit the conformational change that renders the binding site inaccessible to the other molecule.

In view of the protective effect of nucleoside triphosphates against rifampicin inhibition in the DNA-directed reaction, it is surprising that a similar protection is not accomplished by oligomers in the poly U directed reaction. Both before and during synthesis, the sensitivity of the initiation reaction to rifampicin is not diminished by the presence of $A(pA)_4$. Even preliminary incubation of RNA polymerase, poly U, $A(pA)_4$, and manganese does not prevent the rifampicin inhibition. Since the role of the oligomers is presumably that of an initiator, it would seem that an initiation complex between enzyme, template, and oligomer should form. Addition of rifampicin before the oligomer prevents the utilization of the oligomer as an initiator, perhaps by preventing the formation of this initiation complex as a result of some structural change of the enzyme site. However, addition of rifampicin *after* the addition of the oligomer and the presumed formation of the initiation complex still prevents the utilization of oligo A as an initiator. Thus, either the oligomer does not compete at the same site as rifampicin and nucleoside triphosphates or it does not elicit the conformational change that renders the enzyme insensitive to rifampicin. Alternatively, perhaps the oligomer is displaced by rifampicin. Further experimentation is necessary to distinguish between these possibilities. However, the conclusion can be made that oligomers are not capable of bypassing the initiation phase of poly U directed AMP incorporation in the presence of rifampicin.

Thus, while the results presented here confirm both the inhibition of initiation by rifampicin and the stimulatory effect of oligo A on poly U directed poly A synthesis, our data shows that rifampicin inhibits synthesis even in the presence of oligomers. These results suggest that if an initiation complex exists between RNA polymerase, poly U, and $A(pA)_4$, it must differ considerably from the initiation complex formed between RNA polymerase, DNA, and purine nucleoside triphosphates.

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