Mechanistic Aspects of Tagetitoxin Inhibition of RNA Polymerase from *Escherichia coli*†

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ABSTRACT: Tagetitoxin inhibits RNA synthesis directed by bacterial RNA polymerase, and the current study explores several mechanistic aspects of this inhibition. Tagetitoxin inhibition of *in vitro* RNA synthesis directed by *Escherichia coli* RNA polymerase is independent of the template DNA concentration. The toxin can block *Escherichia coli* RNA polymerase during elongation of a nascent RNA chain. In abortive initiation assays, the rate of dinucleotide formation is inhibited by tagetitoxin when initiated with ATP or CpA but not when AMP is used to initiate. Formation of longer oligonucleotides is inhibited by the toxin regardless of the initiating nucleotide. These abortive initiation studies indicate that tagetitoxin does not affect nucleotide substrate binding or phosphodiester bond formation and suggest that the toxin may interfere with a subsequent step. It is suggested that tagetitoxin affects the stability of nascent oligonucleotide binding and/or the translocation of the catalytic center with respect to the 3′-OH of nascent oligonucleotides.

Tagetitoxin is produced by the plant pathogenic bacterium *Pseudomonas syringae* pv. *tagetis* (Mitchell & Durbin, 1981; Mitchell et al., 1989). The toxin prevents chlorophyll accumulation in plants which results in chlorotic symptoms. It has been shown, however, that tagetitoxin does not interfere with chlorophyll biosynthesis *per se* (Lukens, 1983). Rather, the lack of chlorophyll accumulation in tagetitoxin-treated plants appears to be the result of failed chloroplast development (Jutte & Durbin, 1979; Lukens & Durbin, 1985). Tagetitoxin has been shown to inhibit chloroplast RNA polymerase *in vitro* as well as *in organello*, and it has been proposed that this activity accounts for the arrested chloroplast development observed *in vivo* (Mathews & Durbin, 1990). In addition to inhibiting chloroplast RNA polymerase, tagetitoxin was shown to inhibit *in vitro* RNA synthesis directed by RNA polymerase from eubacteria (Mathews & Durbin, 1990) and nuclear RNA polymerase III from several eukaryotes (Steinberg et al., 1990). Nuclear RNA polymerases I and II, on the other hand, appeared to be much less sensitive to the toxin, and RNA synthesis directed by RNA polymerase from bacteriophage T7 or SP6 was unaffected by the toxin (Mathews & Durbin, 1990).

The group of RNA polymerase enzymes sensitive to tagetitoxin is unique and suggests that the toxin may interact with these enzymes at a highly conserved site. Sequence analysis has revealed considerable amino acid conservation among homologous RNA polymerase subunits from very different organisms (Rowland & Glass, 1990). The sequence data indicate that DNA-dependent RNA polymerases can be classified into two broad groups. The first group is homologous to bacteriophage-encoded monomeric enzymes and includes mitochondrial RNA polymerase. The second group consists of multimeric enzymes as found in archaebacteria, eubacteria, chloroplasts, and the eukaryotic nucleus. The RNA polymerase enzymes sensitive to tagetitoxin are all from this second group.

In order to determine how tagetitoxin interferes with RNA synthesis, we investigated several mechanistic aspects of tagetitoxin inhibition of *Escherichia coli* RNA polymerase. RNA synthesis directed by DNA-dependent RNA polymerase is a multistep process (McClure, 1985; von Hippel et al., 1984; Wu & Tweedy, 1982) which involves DNA binding, promoter localization, melting of the DNA double helix to form an open complex, nucleotide substrate binding, phosphodiester bond formation, processive elongation/translocation, and release of the RNA at termination sites. Interference with any of these steps could limit the rate of RNA synthesis. Accordingly, RNA synthesis inhibitors can have very different mechanisms of action (Sarin & Gallo, 1980). For example, some inhibitors bind the DNA template and interfere with the binding or procession of RNA polymerase; others interact directly with the RNA polymerase to prevent DNA binding; some compete with nucleotides for binding to the enzyme; and still other inhibitors seem to specifically interfere with phosphodiester bond formation or translocation.

We present evidence that tagetitoxin is capable of inhibiting the elongation phase of RNA synthesis by interacting with the ternary complex which contains the RNA polymerase core enzyme, the DNA template, and the nascent RNA. Tagetitoxin does not appear to directly interfere with the earlier steps of open complex formation, substrate binding, and phosphodiester bond formation. We speculate that the toxin may interfere with nascent oligonucleotide binding and/or translocation of nascent oligonucleotides with respect to the catalytic active center.

EXPERIMENTAL PROCEDURES

Tagetitoxin was purified from culture filtrates of *Pseudomonas syringae* pv. *tagetis* as described previously (Lukens & Durbin, 1985). *E. coli* RNA polymerase (σ-70 holoenzyme) and bacteriophage T4 DNA were generously provided by C. Gross (University of Wisconsin, Madison, WI). Bacteriophage λ DNA (890P_RPRM) fragment was generously provided by T. Record (University of Wisconsin, Madison, WI). Bacteriophage T7 DNA was prepared essentially by procedures used for purifying bacteriophage λ DNA. The nucleotides ATP, UTP, and CpA used in abortive initiation reactions

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were ultrapure grade from Pharmacia. Other unlabeled nucleotides were from Sigma, and [α-^{32}P]UTP was from Amersham. Bovine serum albumin (BSA) fraction V was from Miles Laboratories. Diethylaminoethyl filters (DE81) and 3MM paper were from Whatmann.

In Vitro Transcription. *In vitro* transcription reactions with *E. coli* RNA polymerase were carried out as previously described (Mathews & Durbin, 1990) using a fixed concentration of tagetitoxin (1 μM) over a range of template DNA concentrations (1–20 μg/mL).

Single-Round Transcription Assays. Single-round transcription assays were carried out essentially as described by Chamberlin and co-workers (1979). Incorporation was monitored by removing 10-μL aliquots periodically and spotting on DE81 filters which had been prespotted with 15 μL of 50 mM sodium pyrophosphate. The filters were then processed to remove unincorporated nucleotides as described (Mathews & Durbin, 1990). To determine whether tagetitoxin could inhibit the transcription complex during the elongation phase, the reaction mix was divided 3.5 min after the reaction was started and combined with tagetitoxin (to a final concentration of 50 μM) or an equal volume of water.

Abortive Initiation Assays. Abortive initiation reactions were carried out according to the method described by McClure et al. (1978). The DNA template, 890P_RPRM, consisted of an 890-bp *Hae*III fragment of bacteriophage λ DNA containing the right promoter (P_R) (Roe et al., 1984). Reaction mixtures contained 40 mM Tris-HCl (pH 8.0); 80 mM KCl; 10 mM $MgCl_2$; 1 mM DTT; 0.05 mM [α-^{32}P]UTP (100 μCi/mL); 0.5 mM ATP, 2.5 mM AMP, or 0.5 mM CpA; 0.64 μg/mL DNA template; 22.5 μg/mL *E. coli* RNA polymerase (σ-70 holoenzyme); and 0, 1, 10, or 100 μM tagetitoxin. After a 10-min preincubation of the enzyme and template at 37 °C, reactions were started by adding the nucleotides. Reactions were incubated for 15 min at 37 °C before samples were spotted onto 2 × 23 cm strips of Whatmann 3MM paper prespotted with 0.1 M EDTA. The paper strips were developed by ascending chromatography in a solution consisting of water/saturated ammonium sulfate/isopropyl alcohol (18:80:2) and 5 mM EDTA. Dinucleotide and oligonucleotide products were distinguished from unincorporated UTP by differences in relative migration (R_f) in this solvent system. The chromatograms were cut into sections, and the radioactivity was measured by counting Cerenkov radiation with a Beckman LS3801 scintillation counter. Unincorporated UTP (R_f = 0.81) was easily distinguished from the dinucleotides pppApU (R_f = 0.44) and pApU (R_f = 0.31) and longer oligonucleotides which do not migrate far from the origin in this solvent system.

RESULTS

DNA Template Concentration. If tagetitoxin interfered with transcription by binding to the DNA template, then its effect would be expected to diminish with increasing DNA concentrations. When a fixed concentration (1 μM) of tagetitoxin was present during *in vitro* transcription reactions directed by *E. coli* RNA polymerase, the extent of inhibition of RNA synthesis remained relatively constant over a range of DNA concentrations (Figure 1). This was the case even when the DNA concentration was increased to levels which were no longer rate limiting.

Tagetitoxin Dilution. An experiment was performed to get some indication of the reversibility of tagetitoxin binding to RNA polymerase. If tagetitoxin bound irreversibly to RNA polymerase, then it should not be possible to dilute the effective

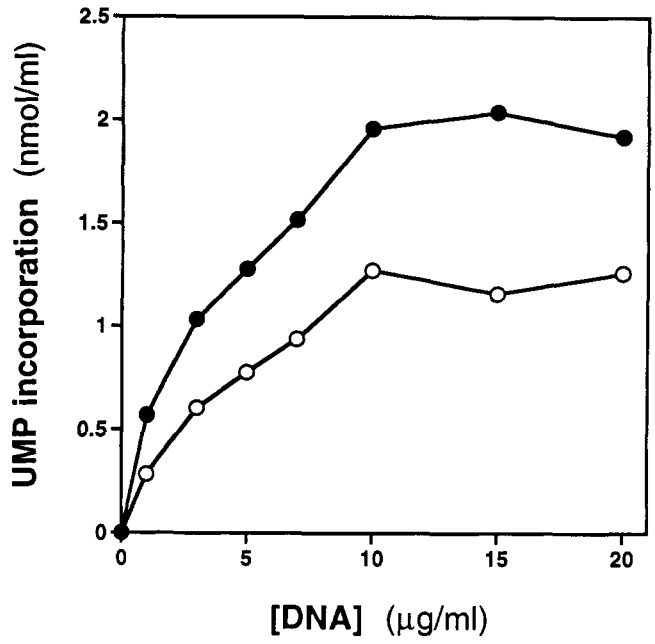


FIGURE 1: Effect of DNA template concentration on tagetitoxin inhibition of *in vitro* RNA synthesis. *In vitro* transcription reaction mixtures directed by purified *E. coli* RNA polymerase contained 0.2 mM each CTP, GTP, and ATP; 0.05 mM [α-^{32}P]UTP (0.1 mCi/mL); and variable concentrations (1–20 μg/mL) of bacteriophage T4 DNA. Reaction mixtures were incubated for 15 min at 37 °C, and the reactions were then stopped by addition of EDTA to a final concentration of 100 mM. Incorporation of [α-^{32}P]UMP into RNA was determined by spotting aliquots onto DE81 filters and measuring the amount of radioactivity that remained bound after washing. Incorporation in control reactions containing no tagetitoxin (●) was compared to incorporation in reactions containing 1 μM tagetitoxin (○).

concentration of tagetitoxin after an initial preincubation at a higher concentration. It was shown by using *in vitro* transcription reactions that when the concentration of tagetitoxin was 0.5 or 10 μM, incorporation was limited to 73% or 14% of control, respectively. When tagetitoxin was combined with RNA polymerase during a preincubation period at a toxin concentration of 10 μM and was then diluted to 0.5 μM simultaneously with the initiation of transcription, the resulting incorporation was 69% of control. Thus, the effective concentration of the toxin during the incorporation reaction was close to the actual diluted concentration and not the preincubation concentration.

Single-Round Transcription Assays. If tagetitoxin were capable of blocking the elongation phase of RNA synthesis, then even incorporation into nascent transcripts would cease during *in vitro* transcription reactions. In order to test this, a single-round transcription assay was used. In this assay, *E. coli* RNA polymerase was combined with bacteriophage T7 DNA and allowed to initiate transcription from the three closely spaced promoters of the bacteriophage T7 "early" region. Reinitiation was prevented by adding heparin 1.5 min after the transcription reaction was started. Heparin binds to free RNA polymerase, preventing the polymerase from binding DNA and subsequently initiating transcription, but it does not interfere with nascent RNA chain elongation. In control reactions without tagetitoxin, there was a period of linear incorporation of nucleotide substrate into RNA as the active enzyme–template complexes transcribed the early region of the bacteriophage T7 genome (Figure 2). Approximately 7 min after transcription initiation, the rate of [α-^{32}P]UTP incorporation decreased, indicating that the transcription complexes had reached the termination signal

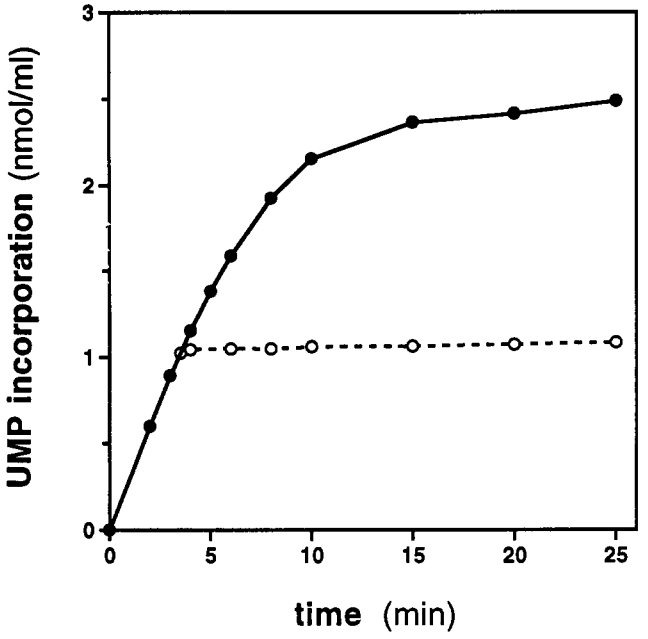


FIGURE 2: Effect of tagetitoxin on RNA chain elongation during a single-round transcription reaction. *E. coli* RNA polymerase was used to transcribe the bacteriophage T7 early region. Transcription initiation occurred at three closely spaced promoters, and reinitiation was prevented by adding heparin to 100 μg/mL at 1.5 min. Samples were removed at intervals, and the incorporation of [α-^{32}P]UMP into RNA was determined by measuring the radioactivity that remained bound to DE81 filters after washing. At 3.5 min (during elongation of the early region transcript) the reaction mixture was divided and combined with either water (●) or tagetitoxin (○) to a final concentration of 50 μM.

approximately 7000 base pairs downstream from the initiation sites. Incorporation did not completely cease at this point because some of the transcription complexes "read through" the termination signal. When tagetitoxin was added to a final concentration of 50 μM during the elongation phase (3.5 min after the reaction was started), nucleotide substrate incorporation into RNA virtually ceased (Figure 2).

Abortive Initiation Assays. To determine whether tagetitoxin could inhibit initiation and formation of the first phosphodiester bond, abortive initiation experiments were performed. *E. coli* RNA polymerase holoenzyme was allowed to bind the bacteriophage λ right promoter (P_R) on the 890P_R-PRM fragment and form an open complex. This promoter uses the sequence 3′ T-A-C-A-T 5′ as the template for the first five nucleotides of the RNA transcript. In the absence of the appropriate nucleotide for the third position, the first dinucleotide formed can be released, and this cycle of nucleotide binding, phosphodiester bond formation, and dinucleotide release is repeated (Johnston & McClure, 1976; McClure et al., 1978).

When the nucleoside triphosphates ATP and UTP were used as the first and second nucleotides, respectively, tagetitoxin reduced formation of the dinucleoside tetraphosphate pppApU (Figure 3A). When the dinucleoside monophosphate CpA was substituted for ATP as the initiating nucleotide, formation of the trinucleoside diphosphate CpApU was also reduced by the toxin (Figure 3B). However, when the nucleoside monophosphate AMP was substituted for ATP, tagetitoxin had much less effect on formation of the dinucleoside diphosphate pApU (Figure 3C). It should be noted that the concentration of AMP used in abortive initiation reactions was 5 times the concentration of ATP or CpA. This concentration was chosen because of the difference in the K_m for AMP as the initiating nucleotide (McClure & Cech, 1978). In separate experiments, it was determined that tagetitoxin did not affect dinucleotide pApU formation over a wide range of AMP concentrations (data not shown).

Addition of the third nucleotide, GTP, does not limit oligonucleotide formation to trinucleotides since the coding sequence of the P_R template begins with 3′ T-A-C-A-T 5′. The products of abortive initiation in this case could be a mixture of oligonucleotides up to 4 or 5 units long, as shown in Scheme 1.

Although addition of GTP significantly reduced the overall rate of UMP incorporation into oligonucleotides when ATP or CpA was used as the first nucleotide (Figure 3A,B),

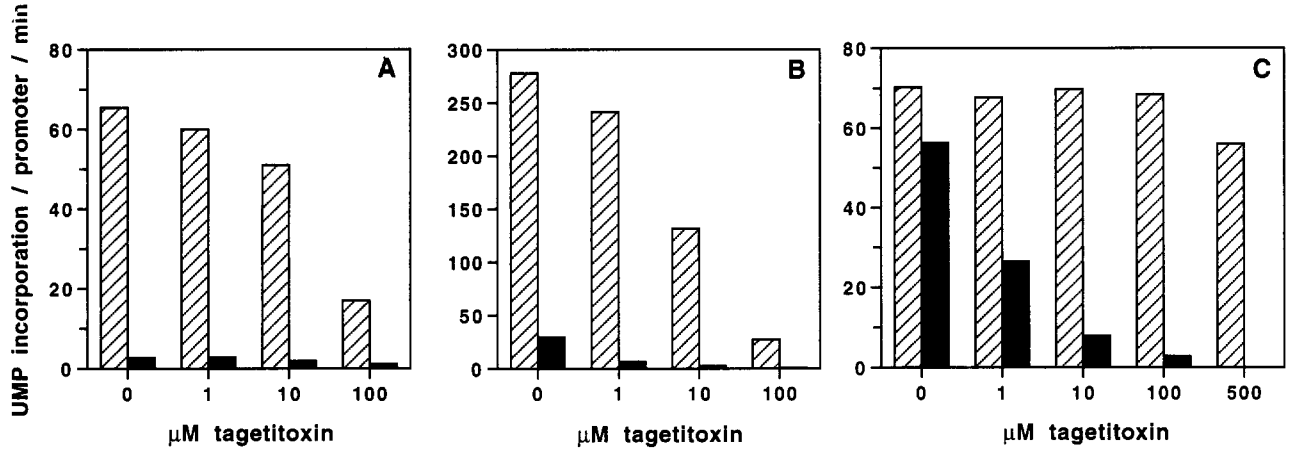
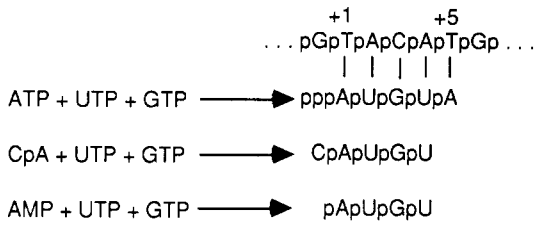


FIGURE 3: Effect of tagetitoxin on formation of dinucleotides and oligonucleotides using the abortive initiation reaction. The incorporation of [α-^{32}P]UMP into dinucleotides or oligonucleotides was determined by product mobility using ascending paper chromatography as described in Experimental Procedures. *E. coli* RNA polymerase, template DNA containing the bacteriophage λ P_R promoter, and tagetitoxin at a final concentration of 0, 1, 10, or 100 μM were preincubated in a reaction mixture for 10 min at 37 °C before nucleotides were added. Tagetitoxin inhibited dinucleotide and longer oligonucleotide formation except for incorporation of UMP into pApU. (A) Addition of 0.5 mM ATP and 0.05 mM [α-^{32}P]UTP resulted in formation of the dinucleotide product pppApU. Inclusion of 0.05 mM GTP allowed formation of oligonucleotides up to 5 nucleotides long (pppApUpGpUpA) (black). (B) Addition of 0.5 mM CpA and 0.05 mM [α-^{32}P]UTP resulted in formatin of the trinucleotide product CpApU (hatched). Inclusion of 0.05 mM GTP allowed formation of oligonucleotides up to 5 nucleotides long (CpApUpGpU) (black). (C) Addition of 2.5 mM AMP and 0.05 mM [α-^{32}P]UTP resulted in formation of the dinucleotide product pApU (hatched). Inclusion of 0.05 mM GTP allowed formation of oligonucleotides up to 4 nucleotides long (pApUpGpU) (black). The mobility profile of α-^{32}P-labeled products suggested that a considerable amount of dinucleotide pApU was formed even when the third nucleotide, GTP, was present.

Scheme 1



tagetitoxin did inhibit the formation of these longer oligonucleotides. Significantly, when GTP was added along with AMP and UTP, tagetitoxin reduced the rate of oligonucleotide synthesis even though there had been little effect on formation of the dinucleoside diphosphate, pApU (Figure 3C). Unlike the situation with ATP or CpA, when AMP was the initiating nucleotide along with UTP and GTP, substantial dinucleotide formation was observed. This dinucleotide formation in the presence of GTP, however, appeared to be inhibited by tagetitoxin.

Kinetics of Inhibition. Abortive initiation reactions were done in which the concentration of ATP was increased from 0.2 to 1.6 mM at a fixed UTP concentration. Since there was no indication that the inhibition of pppApU formation by tagetitoxin could be abolished at elevated ATP concentrations (Figure 4A), tagetitoxin does not appear to compete with the initiating nucleotide for binding to RNA polymerase. When the data are replotted in double-reciprocal form, i.e., (dinucleotide products per minute)$^{-1}$ vs (ATP concentration)$^{-1}$, the plots show that tagetitoxin decreased the V_{max} but did not affect the slope (Figure 4B). The resulting plot best fits the pattern for uncompetitive inhibition.

In another set of abortive initiation reactions, the concentration of the second nucleotide, UTP, was increased from 0.01 to 0.1 mM at a fixed concentration of ATP. There was no indication that tagetitoxin inhibition of dinucleotide formation could be abolished by increasing the UTP concentration (Figure 5A). Replotting the data in double-reciprocal form indicated that tagetitoxin inhibition of dinucleotide formation with respect to the second nucleotide also fit the pattern for uncompetitive inhibition (Figure 5B).

DISCUSSION

Tagetitoxin inhibits *in vitro* RNA synthesis directed by *E. coli* RNA polymerase, and this inhibition cannot be abolished by increasing the DNA template concentration. This result indicates that the toxin interacts with RNA polymerase or the enzyme–template complex but not with the DNA template alone. In addition, the fact that only certain types of RNA polymerase are sensitive to tagetitoxin *in vitro* suggests that the toxin interacts specifically with those enzymes and not in some general way with the DNA template. This selectivity of inhibition would also argue against the possibility that tagetitoxin inhibits RNA synthesis by affecting the stability of nucleotide substrates or product RNA. We conclude, therefore, that tagetitoxin interacts directly with certain RNA polymerase enzymes to inhibit RNA synthesis.

The dose–response curve of tagetitoxin inhibition of RNA synthesis is typical of a simple dissociable enzyme inhibitor. The concentration of toxin required for 50% inhibition of *E. coli* RNA polymerase holoenzyme was about 2 μM, while complete inhibition required almost 100 μM (Mathews & Durbin, 1990). This result is in contrast to the pattern observed for inhibitors such as α-amanitin which bind stoichiometrically (Cochet-Meilhac & Chambon, 1974). Furthermore, since

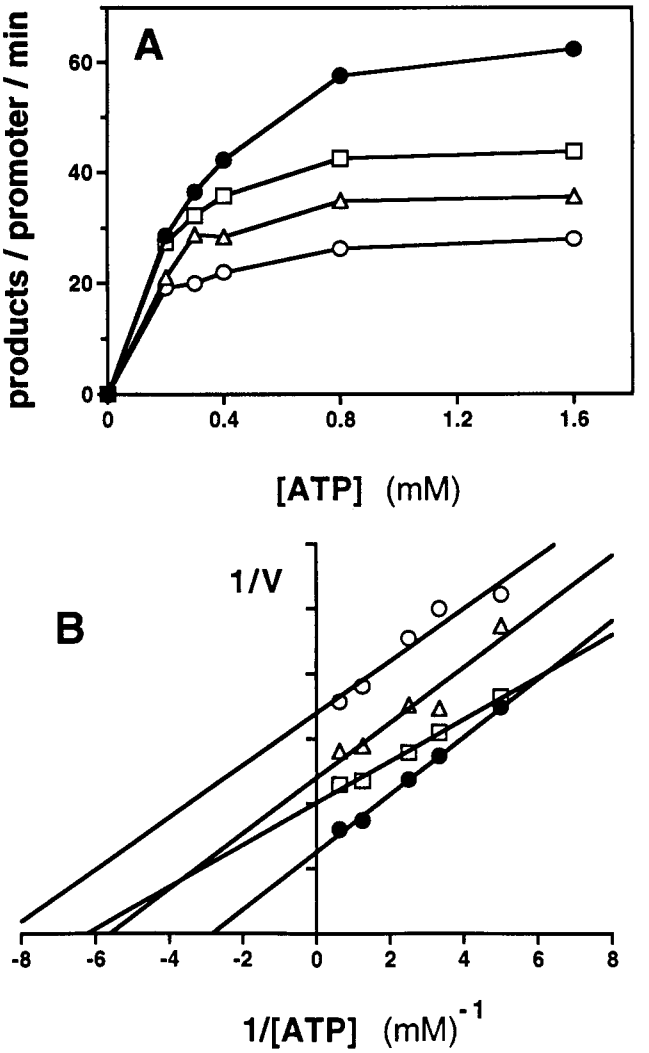


FIGURE 4: Effect of tagetitoxin on ATP saturation of the abortive initiation reaction. (A) The velocity (pppApU products/promoter/min) is plotted vs the ATP concentration for abortive initiation reactions using the phage λ P_R template. The reaction mixtures contained 0.05 mM [α-^{32}P]UTP (0.1 mCi/mL) and 0.2–1.6 mM ATP. Incorporation of [α-^{32}P]UMP into pppApU was determined by ascending paper chromatography as described in Experimental Procedures. The effect on reaction velocity of added tagetitoxin at 5 (□), 20 (△), and 50 μM (○) is compared to controls with no toxin (●). (B) The data from Figure 4A is plotted in double-reciprocal form: (velocity)$^{-1}$ vs (ATP concentration)$^{-1}$. □ The symbols are the same as in panel A.

dilution of the RNA polymerase/toxin solution upon initiation of RNA synthesis resulted in an inhibition rate reflective of the final diluted concentration, it would appear that tagetitoxin can readily dissociate from the enzyme.

Experiments with RNA polymerase III and *E. coli* holoenzyme indicate that tagetitoxin causes enhanced pausing of the transcription complex at discrete sites along the template. This results in an increase in the appearance of low molecular weight, discrete RNAs at the expense of full-length products. The paused complexes were relatively stable, however, and could proceed to form full-length products (Steinberg & Burgess, 1992). Thus, it would appear that tagetitoxin acts as a dissociable inhibitor limiting the overall rate of RNA synthesis.

Using a single-round transcription assay, tagetitoxin was shown to inhibit *E. coli* RNA polymerase during the elongation phase of RNA synthesis. The toxin, therefore, can affect the ternary complex consisting of RNA polymerase core enzyme,

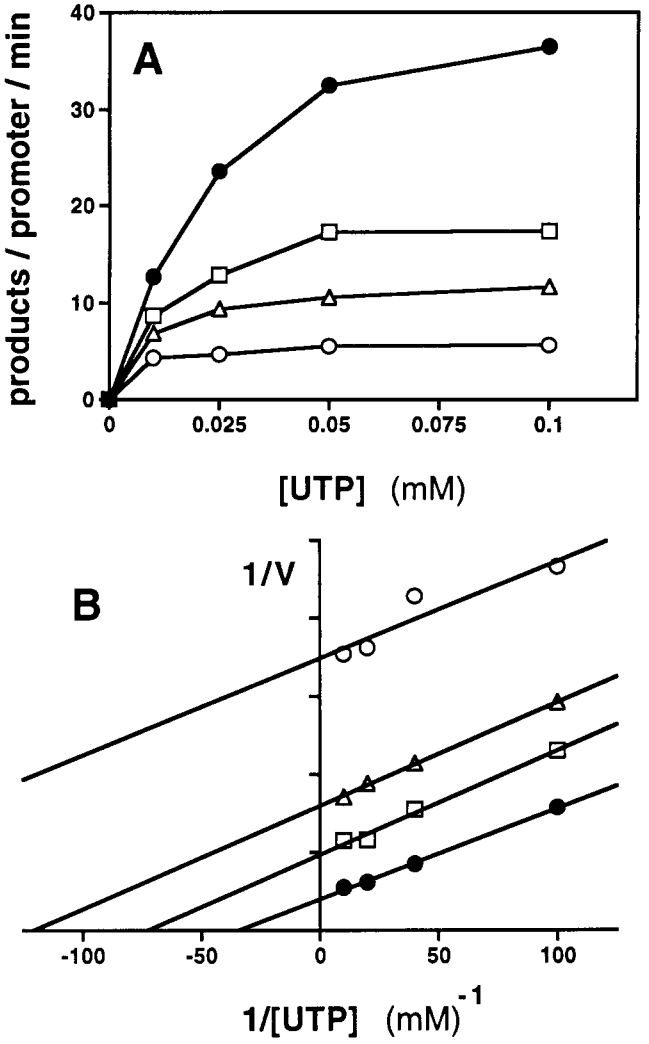


FIGURE 5: Effect of tagetitoxin on UTP saturation of the abortive initiation reaction. (A) The velocity (pppApU products/promoter/min) is plotted vs the UTP concentration for abortive initiation reactions using the phage λ P_R template. The reaction mixtures contained 0.5 mM ATP and 0.01–0.1 mM [α-^{32}P]UTP at constant specific activity. Incorporation into pppApU was determined by ascending paper chromatography as described in Experimental Procedures. The effect on reaction velocity of added tagetitoxin at 5 (□), 20 (△), and 50 μM (○) is compared to controls with no toxin (●). (B) The data from Figure 5A is plotted in double-reciprocal form: (velocity)$^{-1}$ vs (UTP concentration)$^{-1}$. □ The symbols are the same as in panel A.

DNA template, and nascent RNA chain. This result distinguishes tagetitoxin from inhibitors of DNA binding such as heparin and the initiation inhibitor rifampicin. These inhibitors are no longer effective after the RNA polymerase has bound a DNA template and initiated a nascent RNA chain (Fuchs et al., 1967; Sippel & Hartmann, 1968). In addition, preincubation studies with tagetitoxin indicated that the toxin was as effective at inhibiting RNA synthesis when added after DNA binding and open complex formation as it was when added prior to mixing of the RNA polymerase and DNA template (data not shown).

Tagetitoxin inhibited formation of the dinucleoside tetraphosphate pppApU in abortive initiation reactions using phage λ DNA containing the P_R promoter as template along with ATP and UTP. However, when AMP was substituted for ATP, there was little inhibition of dinucleoside diphosphate pApU formation. When GTP was included along with AMP and UTP, synthesis of longer oligonucleotides was inhibited by the toxin much more than pApU formation had been. It is unclear why formation of pApU is largely unaffected by tagetitoxin, but it may represent a steric interaction that results from toxin binding which limits formation of dinucleoside tetraphosphate or longer oligonucleotides but does not affect dinucleoside diphosphate formation.

The effect of tagetitoxin on oligonucleotide formation is reminiscent of the effect of the inhibitor rifampicin. In studies of rifampicin using abortive initiation reactions and the λ P_R template, it was observed that formation of the dinucleoside tetraphosphate pppApU is not prevented, but formation of the trinucleoside pentaphosphate pppApUpG is almost completely blocked by this inhibitor. However, if AMP is substituted for ATP, rifampicin does not prevent formation of the trinucleoside triphosphate pApUpG. It has been proposed that rifampicin sterically interferes with translocation of dinucleoside tetraphosphates such as pppApU, preventing any additional phosphodiester bond formation (McClure & Cech, 1978). According to this model, translocation of dinucleotides with smaller 5′ groups, e.g., ppApU or pApU, is not prevented, yet translocation of any subsequent trinucleotide is prevented. Alternatively, it has been proposed that rifampicin destabilizes the binding of oligonucleotides to the enzyme–template complex (Schulz & Zillig, 1981). According to this scenario, dinucleotides are released from the complex before they can be converted to longer oligonucleotides. Yet, despite some similarities, tagetitoxin binding and inhibition of RNA synthesis is significantly different from the situation with rifampicin. Unlike rifampicin, tagetitoxin can block elongation, and binding is not prevented when nascent RNA occupies a product binding site.

Since RNA synthesis is a complex, multistep process, it is difficult to apply enzyme kinetic analysis. In contrast, dinucleotide formation during abortive initiation reactions is a two-substrate, two-product, steady-state reaction. The inhibition kinetics of tagetitoxin were examined using abortive initiation reactions, and the pattern of inhibition with respect to both the first and the second nucleotide was characteristic of uncompetitive inhibition. Thus, the toxin does not appear to compete with nucleotide substrates for binding to RNA polymerase. Although it was not apparent from the proposed structure (Mitchell et al., 1989) that tagetitoxin could act as a nucleotide analog, this was initially considered a possible mechanism. The uncompetitive inhibition pattern implies that tagetitoxin inhibits an event subsequent to substrate binding; phosphodiester bond formation is such an event. However, the fact that tagetitoxin has little effect on formation of the dinucleotide pApU from AMP and UTP suggests that the toxin does not affect phosphodiester bond formation *per se*. Thus, from data obtained in this study it would appear that tagetitoxin does not specifically interfere with any step of RNA synthesis prior to and including formation of the initial phosphodiester bond.

How else might tagetitoxin inhibit RNA synthesis as well as dinucleoside tetraphosphate formation? Tagetitoxin may affect the binding of oligonucleotides to the enzyme–template complex. If tagetitoxin enhances the binding of dinucleotide tetraphosphates and longer oligonucleotides to the complex, it should reduce the rate of oligonucleotide release and slow the overall rate of product formation. The binding of dinucleoside diphosphates, on the other hand, may be unaffected by the toxin.

Alternatively, it could be proposed that tagetitoxin interferes with translocation of the catalytic active center with respect to the 3′-OH of the nascent oligonucleotide. Such a mechanism would obviously account for the inhibition of nascent RNA

chain elongation, and it may also be consistent with the abortive initiation results if this translocation of the catalytic center is necessary for, or at least facilitates, the release of dinucleotide products from the enzyme–template complex. If translocation of the catalytic center facilitates release of pppApU from the λ P_R template–enzyme complex, then interference with this translocation could explain the reduction of pppApU formation in the presence of tagetitoxin. The smaller dinucleoside diphosphate pApU may be less tightly bound to the enzyme–template complex and may be released without translocation of the catalytic center. The difference in K_m indicates that the affinity of RNA polymerase for AMP is much less than that for ATP when AMP is used as the initiating nucleotide of a dinucleotide product (McClure & Cech, 1978). On the other hand, pApU may avoid some steric interference during translocation of the catalytic center created by enzyme–toxin binding. Such a model would explain why formation of the longer tetranucleotide pApUpGpU, which requires translocation of the catalytic center, would be inhibited by the toxin.

The results of these experiments do not provide a complete explanation of the tagetitoxin mechanism; however, they do allow certain explanations to be discounted. Closer examination of the effect of tagetitoxin on the stability of binding of nascent oligonucleotides to the enzyme–template complex and/or translocation of the catalytic center with respect to the nascent oligonucleotide appears warranted. Since the elongation phase of RNA synthesis is poorly understood, tagetitoxin may prove to be a useful tool in examining RNA polymerase structure-function relationships.

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