Rifampicin as a Spectroscopic Probe of the Mechanism of RNA Polymerase from *Escherichia coli*†

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ABSTRACT: The long-wavelength absorption and circular dichroism (CD) bands of rifampicin and rifampicin quinone have been used to monitor the effects of binding DNA, dinucleotides, and nucleoside triphosphates to RNA polymerase. The consequences of phosphodiester bond formation were also probed. In most of our studies, rifampicin quinone was used because rifampicin was found to undergo rapid autoxidation in Tris buffers. Rifampicin quinone, like rifampicin, forms 1:1 complexes with RNA polymerase and has a K_i of 1.1 μM for the holoenzyme. Changes in the absorption and CD spectra of rifampicin and rifampicin quinone upon binding to RNA polymerase indicate that the antibiotic is bound in a hydrophobic region. The optical properties of the complex were not significantly perturbed by subsequent addition of template [poly[d(A-T)] or poly(dT)], of initiating dinucleotide (ApU or ApA, respectively), or of ATP. Paper chromatographic analysis demonstrated that trinucleotide formation from the dinucleotide and ATP was not inhibited. Nitrocellulose filter binding experiments demonstrated that the absence of a spectroscopic perturbation did not result from failure of the rifampicin quinone–enzyme complex to bind trinucleotides. Our results suggest that the rifampicin quinone binding site is physically removed from the binding site for the RNA product. Hence, the inhibitory effects of rifampicin quinone must result from a conformational change elicited in the enzyme upon binding rifampicin quinone.

Rifampicin and other derivatives of rifamycin SV are potent inhibitors of DNA-dependent RNA synthesis in bacteria (Maggi et al., 1965; Hartmann et al., 1967; Wehrli et al., 1968; Lancini et al., 1969; Wehrli & Staehelin, 1971; Sensi, 1975). These antibiotics bind to RNA polymerase and RNA polymerase–DNA complexes, and until recently, it was thought that rifampicin inhibited initiation. However, Johnston & McClure (1976) and Kessler & Hartmann (1977) have shown that rifampicin does not inhibit the formation of the first phosphodiester bond but prevents the formation of the second phosphodiester bond. When initiating dinucleoside monophosphates are present, even the second phosphodiester bond can be formed in the presence of rifampicin (McClure & Cech, 1978; Oen & Wu, 1978; Sylvester & Cashel, 1980). It has been suggested (Johnston & McClure, 1976; McClure & Cech, 1978) that the inhibitor prevents translocation by steric blockage.

Rifampicin has absorption bands at wavelengths above 310 nm (Maggi et al., 1966) and forms a 1:1 complex with RNA polymerase. The long-wavelength absorption (which does not overlap the intrinsic absorption of RNA polymerase, DNA, or nucleoside triphosphates) and the very tight binding make the antibiotic potentially a good probe of the enzyme structure as well as of other perturbations associated with RNA synthesis. Using the long-wavelength absorption bands of rifampicin (reduced) and rifampicin quinone (oxidized), we have attempted to monitor the effects on RNA polymerase when it binds to poly[d(A-T)], poly(dT), the initiating dinucleoside monophosphates (ApU, ApA), and ATP and the effects on the enzyme when the phosphodiester bond is formed.

Materials and Methods

Rifampicin, poly[d(A-T)], ApA, ApU, and ATP were purchased from Sigma Chemical Co. ApApA and ApApApA were obtained from Boehringer Mannheim. ^{3}H-Labeled ATP was obtained from ICN Chemical and Radioisotope Division. Concentrations were determined by absorption spectroscopy, using the following extinction coefficients: poly[d(A-T)], 6.65 mM^{-1} cm^{-1} at 262 nm (Inman & Baldwin, 1962); poly(dT), 8.1 mM^{-1} cm^{-1} at 260 nm (Bollum, 1966); ApA, 13.0 mM^{-1} cm^{-1} at 260 nm (Powell et al., 1972); ApU, 11.4 mM^{-1} cm^{-1} at 260 nm (Brahms et al., 1967); ATP, 15.4 mM^{-1} cm^{-1} at 259 nm (Pabst Laboratories, 1956); $(Ap)_2A$, 36 mM^{-1} cm^{-1} at 260 nm, and $(Ap)_3A$, 45 mM^{-1} cm^{-1} at 260 nm (Boehringer Mannheim 1980 Catalog). All other chemicals were of reagent grade. The methods for RNA polymerase purification, handling, concentration determination, and assay have been described elsewhere (Reisbig et al., 1979). Circular dichroism (CD),[1] absorption, and UV difference spectra were also obtained according to previously described methods (Reisbig & Woody, 1978; Reisbig et al., 1979). Rifampicin quinone was prepared by oxidation with $K_3Fe(CN)_6$. Rifampicin (500 mg) was dissolved in 50 mL of ethyl acetate. This was then mixed with 150 mL of 20% $K_3Fe(CN)_6$. The aqueous layer was drained, the organic layer was dried over Na_2SO_4, and the ethyl acetate was removed under vacuum. The black crystals were dissolved in chloroform, and the rifampicin quinone was purified by the use of a 1.5 × 60 cm Silicar CC-7 column (Mallinckrodt), using a linear gradient of chloroform (500 mL) to 5% methanol in chloroform (500 mL). The rifampicin quinone was recrystallized from chloroform and hexane. This preparation gave one spot on thin-layer chromatography (TLC) with an R_f of 0.31 in 5% methanol in chloroform, 0.058 in ethyl acetate, and 0.59 in methanol-saturated benzene. There were no other UV- or visible-absorbing spots with any of the three solvents. The UV absorption spectrum showed

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[1] Abbreviations: CD, circular dichroism; TMK buffer, Tris-HCl, pH 7.9, 10 mM $MgCl_2$, and 100 mM KCl; RNase A, ribonuclease A; EDTA, ethylenediaminetetraacetic acid.

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Table I: Extinction Coefficients and Molar Ellipticities for Rifampicin and Rifampicin Quinone

wavelength (nm)	ϵ (mM^{-1} cm^{-1})	$[\theta]$ (deg cm^2 $dmol^{-1}$)
	Rifampicin	
470	15.3	
413		−1500
357		20820
334	26.4	
328		−14870
286		−33300
247		123600
237	33.4	
	Rifampicin Quinone	
560		8300
540	5.36	
420		−3940
354		−26500
332	26.3	
328		−23000
294		−44600
260	26.4	

maxima at 540, 332, and 260 nm [see Maggi et al. (1966) and Table I], and the proton NMR and IR spectra were in agreement with the known structure of rifampicin quinone.

The standard buffer used for spectroscopic studies was 10 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, and 100 mM KCl (to be called TMK buffer).

Nitrocellulose filter binding studies and product analyses were performed as follows. The reaction mixture (55 μL) contained 1.8 μM holoenzyme, 120 μM poly[d(A-T)] [or 95 μM poly(dT)], 1.8 μM rifampicin quinone (for the control, rifampicin quinone was deleted), 90 μM ApU (or 90 μM ApA), and varying amounts of [^{3}H]ATP and ATP. Each component of the reaction mixture was added one at a time with 20-min intervals at 25 °C to simulate the condition of difference spectroscopy. After nucleoside triphosphates were added, the reaction proceeded for 20 min at 25 °C, 10 μL was placed on 3 MM Whatman paper prespotted with 1 mM EDTA, and an additional 25 μL was diluted with 1 mL of TMK buffer for filtration on the nitrocellulose filter.

Analysis of reaction products was performed according to the method of McClure & Cech (1978). The reaction mixture was placed on Whatman 3 MM paper and was developed by ascending chromatography in H_2O–saturated ammonium sulfate–isopropyl alcohol (18:80:2) (solvent I). The chromatogram was air-dried and cut into 1-cm strips except for the origin which was cut from −0.5 to 1 cm and analyzed for radioactivity in toluene-based scintillation fluid. For the verification of the product, the origin was cut and eluted. The eluted material was divided into two parts, and one was subjected to chromatography in 1 M ammonium acetate–95% ethanol (1:1) (solvent II), and the other half was digested with RNase A and subjected to chromatography in solvent II. (We have also used RNase T_2 to identify the product.)

Nitrocellulose filter binding assays were done according to the method of Hinkle & Chamberlin (1972). A 25-μL sample of the final reaction mixture was diluted with 1 mL of TMK buffer and filtered through the nitrocellulose filter with gentle suction. The background count was obtained by mixing enzyme alone with [^{3}H]ATP and treating exactly as described above.

Results and Discussion

The CD and UV spectra of rifampicin and rifampicin quinone are shown in Figure 1. The extinction coefficients and molar ellipticities are presented in Table I. Most of the transitions of rifampicin and rifampicin quinone, except those at wavelengths above 500 nm, are at comparable wavelengths, but the magnitude and in some cases the sign are drastically altered upon oxidation of the antibiotic.

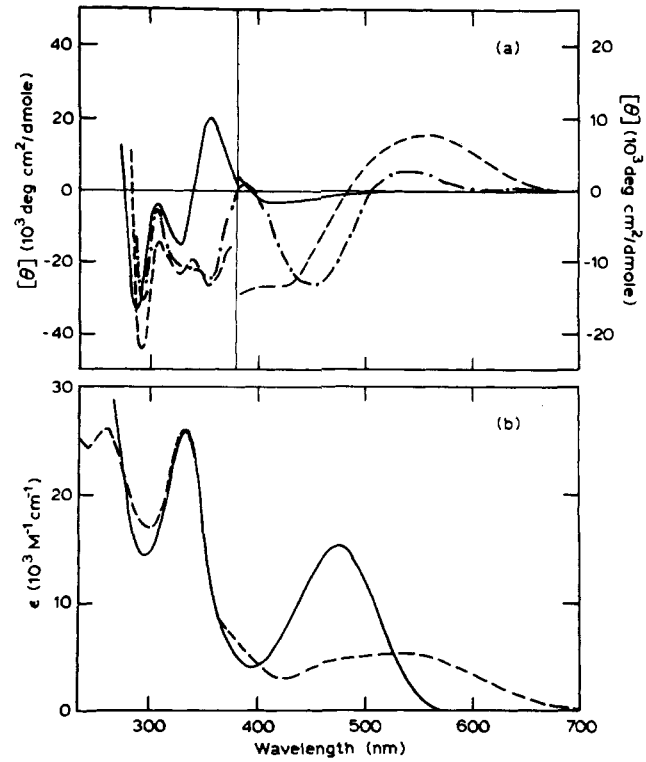

FIGURE 1: (a) CD spectra of rifampicin (—), rifampicin quinone (---), and the rifampicin quinone–RNA polymerase core enzyme complex (-----). Note the change in scale at 380 nm. (b) UV spectra of rifampicin (—) and rifampicin quinone (---). The solvent used was TMK buffer.

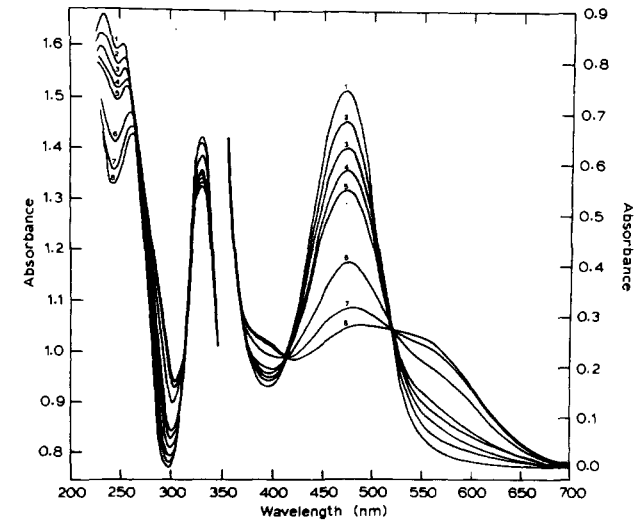

FIGURE 2: Oxidation of rifampicin as measured by the visible and UV spectra. The solvent used was 10 mM Tris-HCl, pH 7.9, 25 °C, with an initial concentration of rifampicin of 48.6 μM. Scans were run at 0 (1), 15 (2), 25 (3), 38 (4), 49 (5), 111 (6), 185 (7), and 259 min (8) after rifampicin was mixed with the buffer.

We have found that when rifampicin is added to Tris buffers there are large changes in the absorption spectrum of the compound over a short period of time. Within 3 h, the absorption spectrum goes from one characteristic of rifampicin to one which is very similar to the oxidized form of the antibiotic, rifampicin quinone (Figure 2). Changes in the CD are also representative of the oxidation of rifampicin. Ri-

fampicin quinone, on the other hand, was stable in buffers without dithiothreitol for up to 24 h.

The sensitivity to oxidation of rifampicin in Tris buffers has allowed us to examine the binding site for rifampicin on RNA polymerase. Rifampicin was added to a molar excess of RNA polymerase, and the absorption spectrum from 700 to 400 nm was monitored. Over a period of 4 h, there was no sign of oxidation, and the absorbance at the 470-nm peak of rifampicin remained constant. This result implies that the naphthodiol nucleus of rifampicin is bound so that it has highly restricted access to the solvent. This observation concurs with the findings of Stender and Scheit (1977) that the binding site for rifampicin is 1.4–1.9 nm from the surface of the enzyme. Our result is in disagreement with the conclusion of Meares & Rice (1981) that the enzyme-bound rifampicin is highly accessible to small molecules in the solvent.

Since the use of rifampicin has been limited by its instability to oxidation, we have turned to the use of rifampicin quinone as a spectroscopic probe of RNA polymerase structure. We have found that rifampicin quinone is a very good inhibitor of RNA polymerase with a K_i (concentration of inhibitor which decreases the enzyme activity by 50%) of 1.1 μM for the holoenzyme. These values agree well with the value reported by Dampier (1975) for rifampicin. While we have not shown directly that rifampicin and rifampicin quinone bind to RNA polymerase in an identical manner, the strong similarities in their structure and inhibition constants and stoichiometry (see below) all support the conclusion that the two forms inhibit RNA polymerase by the same mechanism.

When rifampicin quinone binds to RNA polymerase, the UV difference spectra are characterized by a maximum at 348 nm and a minimum at 318 nm with crossover points at 337 and 302 nm. We observed no difference spectrum at wavelengths above 400 nm. Below 300 nm, a maximum at 290 nm was observed, but due to experimental difficulties (e.g., base-line shifts), the difference spectrum is poorly defined. Comparison of the difference spectrum with the absorption spectrum of rifampicin quinone in TMK buffer (Figures 3a and 1) shows that the absorption maximum at 332 nm is red shifted with a slight increase in intensity upon binding to the enzyme. This implies that the environment in the binding site on the protein is more polarizable than the solvent, consistent with a hydrophobic binding site. Wehrli et al. (1976) have reached a similar conclusion by measuring the K_{eq} for rifampicin binding to RNA polymerase under various solvent conditions. This is also consistent with the protection against oxidation of rifampicin conferred by binding to RNA polymerase.

Using the maximum at 348 nm in the difference spectrum, we have titrated holoenzyme and core enzyme with rifampicin quinone to determine the number of binding sites on the enzyme for the antibiotic (Figure 3B). From Figure 3B, there are 0.90 ± 0.05 and 0.94 ± 0.05 binding sites for the holoenzyme and core enzyme, respectively.

The CD spectrum of rifampicin quinone bound to RNA polymerase shows substantial changes with respect to the CD spectrum of the unbound antibiotic (Figure 1). Changes in both the conformation of the ansa bridge and the environment of the antibiotic could give rise to the observed changes in the CD spectra. At present, we cannot distinguish which, if either, of these effects dominates the observed changes in CD. Within the error limits of our CD measurements ($\simeq$5% where $[\theta] >$ 10000 deg cm^2 dmol^{-1}) the CD spectra of the complexes formed by rifampicin quinone with holoenzyme and core enzyme are identical.

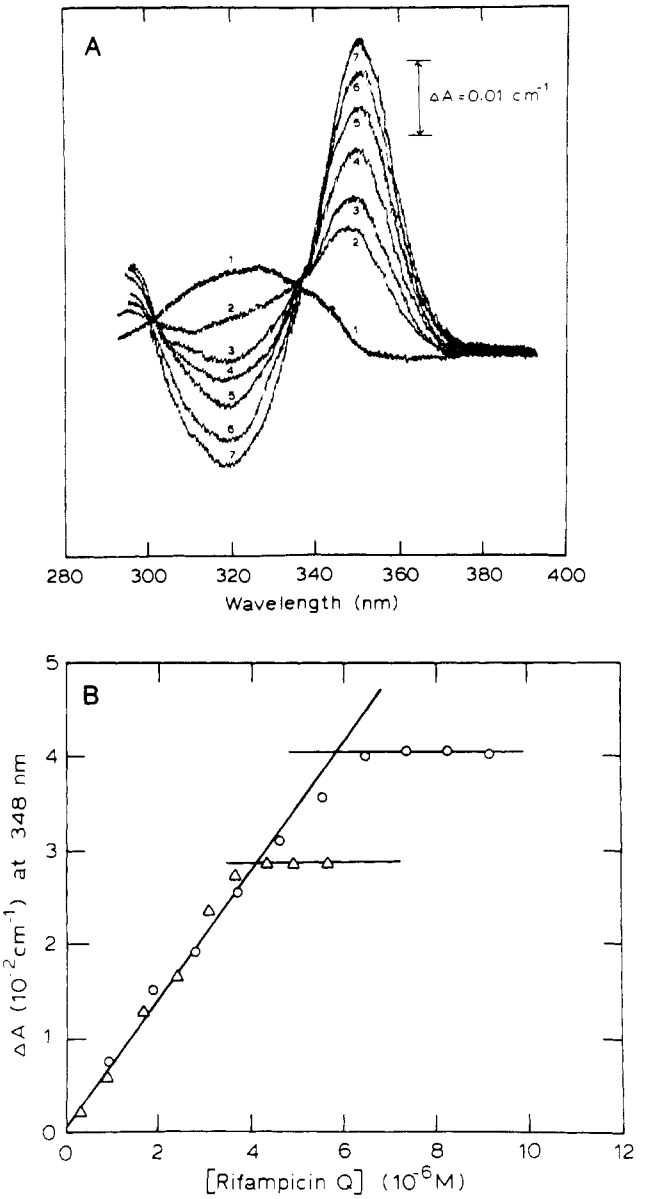

FIGURE 3: (A) UV difference spectra of rifampicin quinone added to RNA polymerase core enzyme in TMK buffer at 25.0 °C. RNA polymerase concentration is 6.2 μM with rifampicin quinone concentrations of 0 M (1), 1.85 μM (2), 2.78 μM (3), 3.70 μM (4), 4.61 μM (5), 5.53 μM (6), and 6.44 μM (7). (B) Titration curve of RNA polymerase holoenzyme and core enzyme by rifampicin quinone monitored at 348 nm. RNA polymerase core enzyme concentration is 6.2 μM (○), and the holoenzyme concentration is 4.6 μM (△).

Wehrli et al. (1976) and Bähr et al. (1976) have shown that holoenzyme and core enzyme have different binding constants for rifampicin under conditions identical with ours. The spectroscopic approach used here may not be sensitive to small changes in the binding site for the antibiotic, especially if such changes affect primarily interactions with the ansa bridge, rather than the naphthoquinone chromophore. From the CD and UV difference spectra, we conclude that the σ subunit does not drastically alter the binding site for rifampicin on core polymerase.

The addition of poly(dT) (134 μM) to the rifampicin quinone–core polymerase complex (6.2 μM) did not alter the CD spectrum of the rifampicin quinone complex. Addition of ApA or ATP also had no effect. Similarly, we did not observe any changes in the CD spectrum of the rifampicin quinone complex with quinone–holoenzyme–poly(dT) holoenzyme upon the addition of poly(dT), ApA, and ATP. The double-stranded

Table II: Chromatographic and Nitrocellulose Filter Binding Analyses[a]

system	chromatographic analysis (1)[b] ApUpA (μM)	(Ap)$_2$A (μM)	(Ap)$_n$A (μM) ($n \geq 3$)	chromatographic analysis (2)[c] ApUpA (μM)	filter binding analysis, radioactivity retained (μM) (1)[b]	(2)[c]
E-poly[d(A-T)]-ApU	2.7			4.5	1.0	0.8
E-poly[d(A-T)]-Rif-ApU	2.7			5.3	0.6	0.6
E-poly(dT)-ApA		1.3	1.3		2.3	6.8
E-poly(dT)-Rif-ApA		1.3	1.3		2.2	6.8

[a] See Materials and Methods for details. [b] 3 μM[^{3}H]ATP (29.8 Ci/mmol). [c] 6.8 μM[^{3}H]ATP (29.8 Ci/mmol).

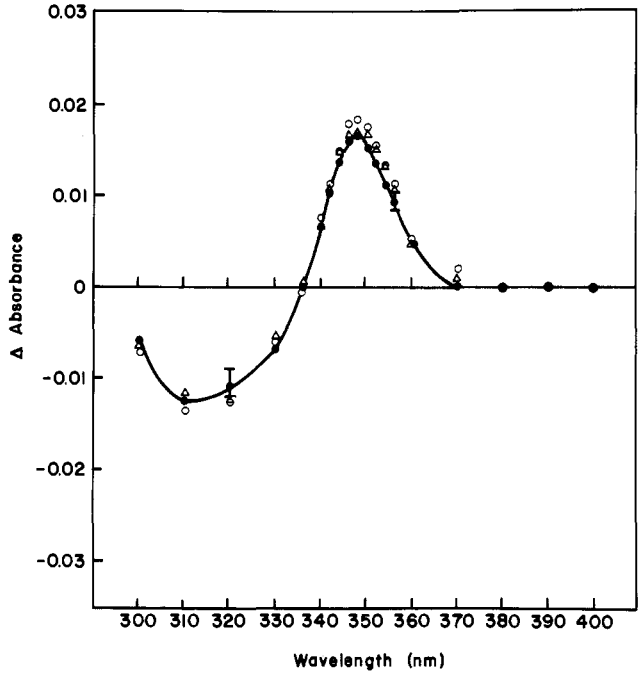

FIGURE 4: UV difference spectra of the rifampicin quinone–holoenzyme–poly[d(A-T)] complex with ApU and ATP. (●) Rifampicin quinone–holoenzyme–poly[d(A-T)] complex; (○) rifampicin quinone–holoenzyme–poly[d(A-T)] complex with added ApU; (△) rifampicin quinone–holoenzyme–poly[d(A-T)] complex with added ApU and ATP. The concentrations used were the following: rifampicin quinone, 2.0 μM; holoenzyme, 2.0 μM; poly[d(A-T)], 132 μM; ApU, 85 μM; ATP, 100 μM.

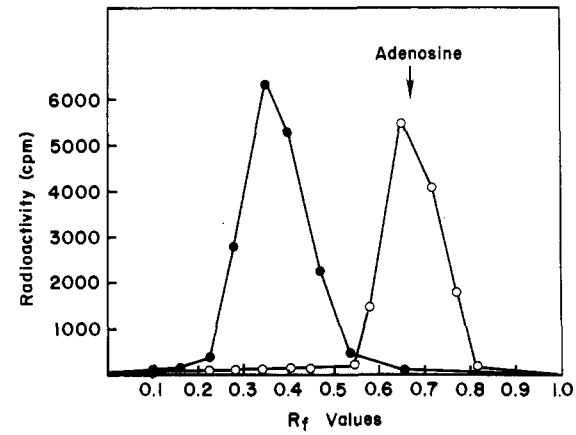

FIGURE 5: Chromatography (3 MM) in solvent II. The reaction mixture containing holoenzyme (1.8 μM), poly[d(A-T)] (120 μM), rifampicin quinone (1.8 μM), ApU (90 μM), and [^{3}H]ATP (3.0 μM) was first chromatographed in solvent I. 91% of the radioactivity was near the origin (–0.5 to 1 cm cut). The material at the origin was divided into two parts—one used for control and the other digested with RNase A at pH 4.5 (15 min at 37 °C). These two solutions were chromatographed in solvent II. Control (●); RNase digest (○).

polynucleotide poly[d(A-T)], when added to the rifampicin quinone–holoenzyme complex (2.6 μM), made no changes in the CD spectrum, and no change was observable when ApU and ATP (84 μM) were added.

The UV difference spectrum of the rifampicin quinone–holoenzyme–poly[d(A-T)] complex is shown in Figure 4. Subsequent addition of ApU and ATP did not perturb the spectrum (see Figure 4). Neither was there any spectral perturbation by the addition of ApA followed by ATP to the rifampicin quinone–holoenzyme–poly(dT) complex. There was a slight increase in light scattering when ATP was added, and this was corrected for by using the empirical equation $A = \text{Const}/\lambda^a$ (Leach & Scheraga, 1960) (data not shown).

To ascertain that a phosphodiester bond is formed between an intiating dinucleotide and nucleotides, under our experimental conditions, we analyzed chromatographically the reaction mixture containing ApU, ATP, and the enzyme–poly[d(A-T)]–rifampicin complex. Over 90% of the radioactive material was near the origin when analyzed in solvent I. Rechromatography of this material in solvent II produced one single peak which was assumed to be ApUp*A. RNase A treatment of this material produced a single peak which coincided with the adenosine peak, confirming that ApUp*A is formed under our experimental conditions (Figure 5). Ribonuclease T_2 treatment of the reaction product confirms this result (data not shown). Rifampicin quinone had no inhibitory effect on the production of ApUpA from ApU and ATP. The concentration of ApUpA produced exceeds the concentration of enzyme in both the presence and absence of rifampicin quinone, suggesting that the enzyme is acting catalytically.

Under our experimental conditions, a concentration of the product ApUpA corresponding to 50% of the enzyme concentration was retained on the nitrocellulose filter in the absence of rifampicin quinone while 35% was retained in the presence of rifampicin quinone. Thus, rifampicin quinone destabilizes the ternary complex of enzyme–polynucleotide–ApUpA to some extent, but still a significant amount of ApUpA is in the form of the ternary complex, even if the enzyme is acting catalytically. With poly(dT) as a template, the effect of rifampicin quinone is much less on the ternary complex. These results are presented in Table II.

The production of ApUpA on poly[d(A-T)] template with ApU and ATP has been reported previously by Oen & Wu (1978) and Sylvester & Cashel (1980). Oen and Wu observed catalytic production of ApUpA, from which they concluded that this trinucleotide does not form a stable ternary complex. According to Sylvester and Cashel, the production of ApUpA is stoichiometric in the absence of rifampicin while it is catalytic in the presence of rifampicin. They infer from the catalytic nature of ApUpA production determined by gel filtration and Millipore filter binding studies that ApUpA does not form a stable ternary complex in the presence of rifampicin. The disagreement may stem in part from the different experimental conditions used. The conditions for the Millipore filter binding studies used by Sylvester and Cashel are quite

different from the conditions we have used here. Also, in our experiment the concentration of rifampicin quinone employed was equal to that of the enzyme. The very high association constant of the enzyme–rifampicin complex ($K_s = 3 \times 10^9$ M^{-1}) (Bähr et al., 1976; Wehrli et al., 1976) assures nearly complete binding of the inhibitor to RNA polymerase under our conditions. However, normally in inhibition studies the level of rifampicin used is much higher than that of enzyme, and the large excess may lead to occupation of weaker binding sites.

McClure & Cech (1978) have recently proposed a model in which rifampicin inhibition is due to steric blockage of translocation after dinucleoside tetraphosphate formation or trinucleotide formation if the initiating dinucleotide lacks a 5′-triphosphate. This suggests that the rifampicin binding site is in the proximity of the product binding site. If this were the case, one would expect to see perturbations in the CD and difference spectra from the coupling of the electronic transitions of the bases of the product and those of rifampicin quinone. Our failure to observe such spectral perturbations has three possible interpretations: (1) The rifampicin quinone binding site is physically removed from the binding site of the RNA product, and the inhibitory effect of rifampicin is through a conformational change elicited in the enzyme. Wu & Wu (1974) have concluded from energy transfer studies that rifampicin is ca. 34 Å from the active site of RNA polymerase. Our observations are consistent with this conclusion. (2) The synthetic process does not produce conformational changes in the enzyme near the binding site of rifampicin quinone. (3) Hindrance of translocation occurs via the ansa bridge, and the naphthoquinone chromophore is not affected by oligonucleotide synthesis. Such hindrance by an ansa bridge might not preclude the synthesis of shorter oligomers but could block the synthesis of longer oligomers.

References

Bähr, W., Stender, W., Scheit, K., & Jovin, T. M. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 369–396, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Bollum, F. J. (1966) in *Procedures in Nucleic Acid Research* (Cantoni, G. L., & Davies, D. R., Eds.) pp 577–583, Harper and Row, New York.

Brahms, J., Maurizot, J. C., & Michelson, A. M. (1967) *J. Mol. Biol. 25*, 481–495.

Dampier, M. F. (1975) Ph.D. Thesis, University of Wisconsin, Madison, WI.

Hartmann, G. R., Honikel, K. O., Knüsel, F., & Nüsch, J. (1967) *Biochim. Biophys. Acta 145*, 843–844.

Hinkle, D. C., & Chamberlin, M. J. (1972) *J. Mol. Biol. 70*, 157–185.

Inman, R. B., & Baldwin, R. L. (1962) *J. Mol. Biol. 5*, 172–184.

Johnston, D. E., & McClure, W. R. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 413–428, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Kessler, C., & Hartmann, G. R. (1977) *Biochem. Biophys. Res. Commun. 74*, 50–56.

Lancini, G., Pallanza, R., & Silvestri, L. G. (1969) *J. Bacteriol. 97*, 761–768.

Leach, S. J., & Scheraga, H. A. (1960) *J. Am. Chem. Soc. 82*, 4790–4792.

Maggi, N., Pallanza, R., & Sensi, P. (1965) *Antimicrob. Agents Chemother. (1961–70)*, 765–769.

Maggi, N., Pasqualucci, C. R., Ballota, R., & Sensi, P. (1966) *Chemotherapia 11*, 285–292.

McClure, W. R., & Cech, C. L. (1978) *J. Biol. Chem. 253*, 8949–8956.

Meares, C. F., & Rice, L. S. (1981) *Biochemistry 20*, 610–617.

Oen, H., & Wu, C.-W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1778–1782.

Pabst Laboratories (1956) *Circular OR-10*, p 21.

Powell, J. T., Richards, E. G., & Gratzer, W. B. (1972) *Biopolymers 11*, 235–250.

Reisbig, R. R., & Woody, R. W. (1978) *Biochemistry 17*, 1974–1984.

Reisbig, R. R., Woody, A-Y. M., & Woody, R. W. (1979) *J. Biol. Chem. 254*, 11208–11217.

Sensi, P. (1975) *Pure Appl. Chem. 41*, 15–29.

Stender, W., & Scheit, K. H. (1977) *Eur. J. Biochem. 76*, 591–600.

Sylvester, J. E., & Cashel, M. (1980) *Biochemistry 19*, 1069–1074.

Wehrli, W., & Staehelin, M. (1971) *Bacteriol. Rev. 35*, 290–309.

Wehrli, W., Knüsel, F., Schmidt, K., & Staehelin, M. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61*, 667–673.

Wehrli, W., Handschin, J., & Wunderli, W. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 397–412, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Wu, F. Y-H., & Wu, C.-W. (1974) *Biochemistry 13*, 2562–2566.