Articles

Resonance Energy Transfer Study on the Proximity Relationship between the GTP Binding Site and the Rifampicin Binding Site of *Escherichia coli* RNA Polymerase

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ABSTRACT: Terbium(III) upon complexation with guanosine 5′-triphosphate showed remarkable enhancement of fluorescence emission at 488 and 545 nm when excited at 295 nm. Analysis of the binding data yielded a value for the mean K_d between Tb(III) and GTP of 0.2 μM, with three binding sites for Tb(III) on GTP. ^{31}P and ^{1}H NMR measurements revealed that Tb(III) mainly binds the phosphate moiety of GTP. Fluorescence titration of the emission signals of the TbGTP complex with varying concentrations of *Escherichia coli* RNA polymerase resulted in a K_d value of 4 μM between the TbGTP and the enzyme. It was observed that TbGTP can be incorporated in the place of GTP during *E. coli* RNA polymerase catalyzed abortive synthesis of dinucleotide tetraphosphate at T7A2 promoter. Both the substrate TbGTP and the inhibitor of the initiation of transcription rifampicin bind to the β-subunit of *E. coli* RNA polymerase. This allows the measurement of the fluorescence excited-state energy transfer from the donor TbGTP–RNA polymerase to the acceptor rifampicin. Both emission bands of Tb(III) overlap with the rifampicin absorption, and the distances at 50% efficiency of energy transfer were calculated to be 28 and 24 Å for the 488- and 545-nm emission bands, respectively. The distance between the substrate binding site and the rifampicin binding site on the β-subunit of *E. coli* RNA polymerase was measured to be around 30 Å. This suggests that the nature of inhibition of transcription by rifampicin is essentially noncompetitive with the substrate.

DNA-dependent RNA polymerase from *Escherichia coli* has been the focus of many investigations over the past few years (Losick & Chamberlin, 1976; Kumar, 1981; von Hippel et al., 1984). This is a multisubunit enzyme ($\alpha_2\beta\beta'\sigma$) containing two tightly bound Zn atoms per molecule of protein (Wu & Wu, 1981). Structural analysis carried out to date on this enzyme has revealed the following facts: RNA polymerase from *E. coli* has two substrate binding sites (Wu & Goldthwait, 1969a). One of them is purine nucleotide specific, located at the β'-subunit of the enzyme and is generally template and Mg(II) ion independent (Wu & Goldthwait, 1969b). On the other hand, the other substrate binding site is responsible for the elongation or polymerization of RNA chain and, therefore, Mg(II) ion and template dependent, and located at the β'-subunit of the enzyme (Miller et al., 1979). The former site is known as the initiation site and the latter as the elongation site of the enzyme (Chatterji & Wu, 1982a).

The rifampicin class of antibiotics has been intensively studied since the observation of Sippel and Hartmann that rifampicin inhibited the initiation of RNA synthesis in *E. coli* (Sippel & Hartmann, 1968). Over the years a clear picture on the nature of binding of rifampicin to RNA polymerase has emerged (Bahr et al., 1976; Gurgo, 1980). That is, rifampicin binds strongly to the β-subunit of RNA polymerase, and this site is located very close to the σ-subunit of the holoenzyme. However, some observations made earlier seem to be puzzling. Although it was pointed out that rifampicin inhibits the substrate binding to the initiation site, McClure and Cech (1978) later reported that the major effect of rifampicin is a total block of the translocation step that would oridinarily follow formation of the first phosphodiester bond. Moreover, the effect of rifampicin on the binding of the initiating nucleotide to the RNA polymerase was observed to be competitive in the absence of a template and noncompetitive in the presence of a template DNA (Wu & Goldthwait, 1969b; Anthony et al., 1969). These ambiguities can indeed be resolved if the geometric relationship between the initiation or substrate binding site on the β-subunit of *E. coli* RNA polymerase ans the rifampicin binding site on the same subunit is understood. Fluorescence resonance energy transfer measurements appear to be a potential technique for such purpose, and this has been used in a few cases to monitor the distances among various positions on *E. coli* RNA polymerase (Wu et al., 1976; Chatterji et al., 1986; Wu & Tyagi, 1987). Rifampicin can act as a very good acceptor of the radiation toward a suitable donor emitting in the visible region, which in our case should be an intiating nucleotide. We observed in the past that the Tb(III) ion, otherwise nonfluorescent, shows sensitized luminescence when complexed with guanosine nucleotides (Chatterji, 1986, 1988). Interestingly, the TbGTP emission bands overlap with one of the absorption bands of rifampicin, forming a Förster donor–acceptor pair. In this paper, we report the distance between the TbGTP binding site and the rifampicin binding site on *E. coli* RNA polymerase and the rationale behind such measurements.

Experimental Procedures

Materials

Rifampicin and nucleotides were purchased from Sigma and Boehringer-Manheim, respectively. The quality of the triphosphate was checked by thin-layer chromatography on poly(ethylene imine) (PEI) plates as described before (Schleif & Wensink, 1981). $TbCl_3$ was a product of Aldrich, and its concentration was fixed by fluorometric assay as reported earlier (Barela & Sherry, 1976). All other routine chemicals

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were of the purest grade available. Radionucleotides used for RNA polymerase assay were the product of Amersham.

Methods

E. coli RNA polymerase was purified from mid log phase cells of a RNase I$^-$ strain (MRE 600) essentially following the method of Burgess and Jendrisak (1975) but with a modification developed in this laboratory (Kumar & Chatterji, 1988). Sodium dodecyl sulfate–polyacrylamide gel electrophoretic (SDS–PAGE) analysis showed the enzyme was 98% pure. Steady-state assay for the total RNA synthesis over $\Delta D_{111}T_7$ DNA by this enzyme was followed by monitoring the incorporation of [α-^{32}P]UTP to the RNA product. The enzyme was found to have a specific activity of 2200 units/mg (Kumar & Chatterji, 1988). Heparin-resistant elongation of RNA chain measured over $\Delta D_{111}T_7$ DNA containing a single promoter (T7A1) indicated that the enzyme preparation had about 60% active molecules (Kumar & Chatterji, 1988). *E. coli* RNA polymerase purified in this way was routinely stored in 40 mM Tris-HCl (pH 7.9), 50% glycerol, 0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM dithiothreitol at −20 °C. The protein concentration was determined by absorption spectroscopy using an extinction coefficient (1% ϵ_{280nm}) of 6.2 and a molecular weight of 450 000 (Lowe et al., 1979). The rifampicin concentration was fixed by using the molar extinction value of 28 000 at 334 nm (Bahr et al., 1976).

Abortive initiation of transcription was carried out according to the method described before (Johnston & McClure, 1976; McClure & Cech, 1978). A derivative of *E. coli* strain HMS 174, containing plasmid pAR 1539, which has a single early promoter A2 of bacteriophage T7, was kindly provided by Dr. F. W. Studier of Brookhaven National Laboratory. This plasmid was purified in the usual way and was used as a template for the RNA polymerase catalyzed synthesis of the RNA. Under the conditions of the abortive initiation of transcription at T7A2 promoter with the supply of the first two initiating nucleotides GTP and CTP, only the dinucleotide pppGpC was synthesized in the presence of *E. coli* RNA polymerase. However, when this reaction was carried out with TbGTP, it was incorporated in the place of GTP, and the product, a Tb(III) complex of pppGpC, was separated from the mononucleotides in a Bio-Gel P2 column (0.7 cm × 45 cm) having a 0.5-mL fraction with Tris-HCl (pH 7.5) and 0.1 mM EDTA buffer.

Fluorescence Measurements. All fluorescence experiments were performed in the buffer containing 10 mM Tris-HCl (pH 7.5) and 100 mM KCl. Just before the experiments, the enzyme was routinely dialyzed against the above buffer to minimize the concentration of glycerol, which otherwise interferes with the reproducibility of the Tb(III) emission signal. We also found that the optimum pH of the medium was 7.5 with respect to the enzyme activity and the stability of Tb(III) in the solution. At higher pH Tb(III) tends to precipitate out as $Tb(OH)_3$ on long standing. Complexes between Tb(III) and GTP were prepared by mixing at appropriate pH. Fluorescence measurements were carried out in a Hitachi F-4000 spectrofluorometer with spectral correction. A few uncorrected spectra were also recorded by using a Hitachi 650-10S spectrofluorometer. The absorption of the samples was kept low at the excitation wavelength so as to avoid inner-filter quenching. All spectral measurements were carried out at 24 °C.

Quantum Yields of the Samples. The quantum yield, Q_S, of a sample was calculated from the absorbance (A) and the area enclosed by the corrected emission spectrum by using (Parker & Rees, 1960)

$$Q_S = \frac{Q_R(1 - 10^{-A_R})(\text{area})_S n_R^2}{(\text{area})_R(1 - 10^{-A_S}) n_S^2} \quad (1)$$

where n is the fractive index of the solvent, and S and R refer to the sample and reference, respectively. Both quinine sulfate and fluorescein in H_2SO_4 and NaOH, respectively, were taken as references (Cantor & Schimmel, 1980), and the internal consistency of the result was checked by measuring the quantum yield of one with respect to the other. The areas of the corrected emission spectra were calculated by using the computer paired with the spectrofluorometer.

Measurement of the Distance between the Donor and the Acceptor by the Application of Förster's Theory. In our case the donor was the TbGTP complex of RNA polymerase, and rifampicin was the acceptor. In Förster's theory of dipole–dipole energy transfer (Förster, 1948), the transfer efficiency (T) is related to the distance, r, (angstroms) between the donor and the acceptor according to

$$r = R_0((1/T) - 1)^{1/6} \text{ or } T = r^{-6}/(r^{-6} + R_0^{-6}) \quad (2)$$

T also can be expressed as

$$1 - (Q/Q_0) \quad (3)$$

where Q and Q_0 are the quantum yields of donor in the presence and absence of acceptor.

R_0, the distance (angstroms) at which the transfer efficiency equals 50%, is given by

$$R_0 = 9.79 \times 10^3[(J)Q(n^{-4})(\kappa)^2]^{1/6} \quad (4)$$

where n is the refractive index between donor and acceptor in the medium and was taken as 1.4. J, the spectral overlap integral between the emission spectrum of the donor and the absorption spectrum of the acceptor, was approximated by the summation

$$J\ (\text{M}^{-1}\ \text{cm}^3) = \frac{\int F_d(\lambda)\epsilon_a(\lambda)\lambda^4\, d\lambda}{\int F_d(\lambda)\, d\lambda} \quad (5)$$

where $F_d(\lambda)$ and $\epsilon_a(\lambda)$ are the relative fluorescence intensity (%) of the donor and the molar extinction coefficient (M^{-1} cm^{-1}) of the acceptor, respectively, and λ is the wavelength at nanometer interval, $\Delta\lambda$. A small FORTRAN program written by us for the calculation of J is given in the Appendix.

The major uncertainty with the calculation of R_0 is the orientation factor κ^2, which is usually taken as $^2/_3$. However, if either the donor or acceptor is a metal ion, then because of the degeneracy of the excited state, the κ^2 value of $^2/_3$ is a very good approximation, as in our case, and the maximum error limit in the distance measurement lies within 10% (Stryer, 1978; Holmquist, 1980). One should also keep in mind that the discrepancy in the values of κ^2 or n is not reflected in R_0 as much because of the sixth-root dependence.

NMR Measurements. Both the ^{1}H and ^{31}P NMR measurements were carried out by using a Varian CFT-20 or a Bruker AM-300 NMR machine operating at 80 or 300 MHz, respectively, for proton.

Results

TbGTP Binding to E. coli RNA Polymerase. Although there are various papers available in the literature on the nature of Tb(III) binding with guanosine residue in DNA and RNA (Gross & Simpkins, 1981; Chatterji, 1986, 1988), no quantitation has so far been attempted on the stability of TbGTP by fluorometric assay. We therefore undertook a detailed analysis of Tb(III) binding with GTP using sensitized lanthanide fluorescence. $TbCl_3$ in Tris-HCl buffer (pH 7.5) when

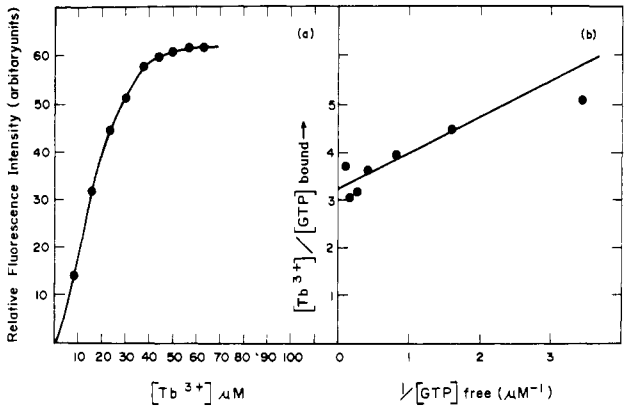

FIGURE 1: (a) Variation in fluorescence emission at 545 nm of TbGTP complex with different concentrations of Tb(III) at a fixed GTP concentration (10 μM). Excitation wavelength is 295 nm. (b) Analysis of the titration curve shown in (a).

excited at 295 nm does not show any appreciable fluorescence in the visible region. However, upon complexation with GTP two major emission peaks appear at 488 and 545 nm. We attempted to quantitate the Tb(III) binding to GTP by titrating the 545-nm emission band with varying Tb(III) concentrations at a fixed concentration of GTP (see Figure 1a). Analysis of the titration data was carried out in the following way (Topal & Fresco, 1980); if there are n number of binding sites available on GTP for Tb(III), then the mean association constant K between Tb(III) and GTP would be

$$K = \frac{[\text{bound residue}]}{(\text{free residue})(n[\text{Tb(III)}] - [\text{bound residue}])} \quad (6)$$

which on rearranging becomes

$$\frac{[\text{Tb(III)}]}{[\text{bound residue}]} = \frac{1}{nK[\text{free residue}]} + \frac{1}{n} \quad (7)$$

Figure 1b shows a plot of the binding data obtained from fluorescence titration which yielded K_d(TbGTP) ($1/K$) of 0.2 μM with n equal to 3. It should be mentioned here that the complexometric titration carried out earlier between various lanthanides and nucleotides estimated a K_d of the same order (Morrison & Cleland, 1983). Similar values for K_d and n were also obtained when the titration was performed with another fixed concentration of GTP. Thus, a dissociation constant value of 0.2 μM with three binding sites indicates an average K_d between Tb(III) and GTP. However, the affinities between each of these sites and Tb(III) may not be very much different from each other if the nature of the coordinating ligands is same. When we recorded the ^{1}H and ^{31}P NMR spectra of GTP and the TbGTP complex, the broadening of all the phosphorus peaks was observed in the complex without much change in the resonance of ring protons (not shown). These experiments indicated Tb(III) binds to GTP mainly through phosphate coordination. It should be mentioned here, although a high concentration of GTP was necessary for clarity of the signals, that 3:1 stoichiometry of the TbGTP complex was maintained as in the fluorescence experiment. However, a 1:1 complex of TbGTP also showed broadening of the phosphorus resonances.

When *E. coli* RNA polymerase was added to the TbGTP (3:1) complex, it was observed that the intensity of the fluorescence emission diminished. As RNA polymerase alone in this concentration range did not induce any enhancement of the emission signals of Tb(III), we propose that the decrease in TbGTP emission signals in the presence of the enzyme is due to the complex formation between TbGTP and RNA

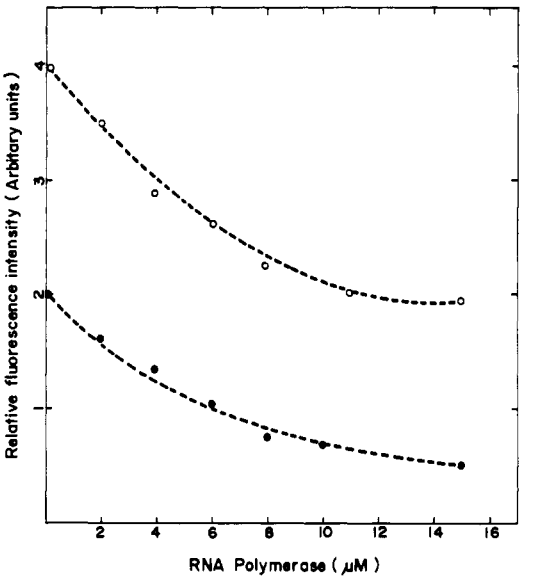

FIGURE 2: Fluorescence quenching titration of TbGTP [3 μM Tb(III) and 1 μM GTP] with varying concentrations of *E. coli* RNA polymerase in 10 mM Tris-HCl (pH 7.5) and 100 mM KCl. (○) 545-nm emission of TbGTP; (●) 488-nm emission band of TbGTP.

polymerase. Figure 2 shows the titration of the emission peaks of TbGTP with varying concentrations of the enzyme. Both curves represent the same complex between the enzyme and TbGTP, and the K_d value of the complex obtained from the half-maximal saturation was 4 μM. Thus, the stability of the complex TbGTP (K_d = 0.2 μM) is much more than that of the second complex between TbGTP and RNA polymerase (K_d = 4 μM), and the dissociation of TbGTP in the presence of the enzyme is expected to be insignificant. However, the value of K_d between purine nucleotides and RNA polymerase was reported to be 150 μM in the absence of a template (Wu & Goldthwait, 1969a; Chatterji et al., 1984). Due to this apparent discrepency in their K_d values, we asked whether TbGTP binds to RNA polymerase at the same site as GTP.

Abortive Initiation of Transcription with TbGTP. To follow the transcription reaction, the plasmid pAR 1539 was used as a template (see Methods). This has a single strong promoter of bacteriophage T7, A2, which was cloned at the *Bam*H1 site of the vector pBR 322. Any transcription catalyzed by *E. coli* RNA polymerase over pAR 1539 would be mainly initiated at the T7A2 promoter giving rise to a message GCUA... (Dunn & Studier, 1983). Thus, in the presence of GTP and CTP, the initiation of transcription would be aborted with the accumulation of pppGpC. It was our interest to find whether TbGTP could be incorporated at the 5′-terminus of the aborted message in the place of GTP. Figure 3 shows the Bio-Gel P2 profile of the transcription product at the T7A2 promoter, in the presence of only TbGTP and radiolabeled CTP. It can be seen from the figure that the faster moving peak, presumed to be a Tb(III) complex of pppGpC, is fluorescent, indicating TbGTP binds the initiation site of *E. coli* RNA polymerase. As the abortive initiation of transcription needs to be carried out at pH 8, we have used a 1:1 TbGTP complex in this case to avoid precipitation of Tb(III).

Binding of Rifampicin with TbGTP–RNA Polymerase. Rifampicin has two absorption peaks, one around 330 nm and the other at the longer wavelength of 470 nm (Bahr et al., 1976). Therefore, in priniciple it can form a Förster's donor–acceptor pair with TbGTP provided the two are located at suitable distances. It was indeed observed that upon addition of riampicin to the TbGTP–RNA polymerase complex both emission bands of TbGTP were quenched as shown in

Table I: Spectral Overlap Integral between the Donor (TbGTP–RNA Polymerase Complex) and the Acceptor Rifampicin, Quantum Yield of the Donor, Energy-Transfer Efficiencies, and Distance between the Donor and the Acceptor in the *E. coli* RNA Polymerase–Rifampicin Complex

sample[a]	emission wavelength (nM)	J (M^{-1} cm^3) (spectral overlap integral)	Q (quantum yield)	R_0 (Å) (distance at 50% energy-transfer efficiency)	T (energy-transfer efficiency)	r (Å) (distance between donor and acceptor)
TbGTP–RNA polymerase	488		0.046			
TbGTP–RNA polymerase + rifampicin	488	6.56×10^{14}	0.034	27.8	0.26	33.1
TbGTP–RNA polymerase	545		0.072			
TbGTP–RNA polymerase + rifampicin	545	1.52×10^{14}	0.058	23.5	0.19	29.9

[a] Energy-transfer measurements were carried out with the 3 μM Tb(III)–1 μM GTP complex bound to 10 μM RNA polymerase and 10 μM rifampicin.

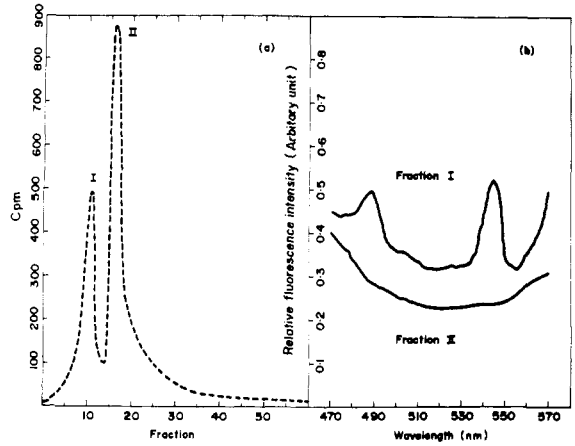

FIGURE 3: Synthesis of Tb(III) complex of pppGpC by *E. coli* RNA polymerase at T7A2 promoter. The reaction was carried out at 37 °C for 30 min in a total mixture of 100 μL containing the plasmid pAR 1539 (T7A2), 0.2 mM DNA-phosphorus, 40 μg/mL RNA polymerase, 5 mM $MgCl_2$, 40 mM Tris-HCl (pH 8), 200 μM GTP, 200 μM Tb(III), and 3 μM [α-^{32}P]CTP (1 μCi/nmol). The reaction volume was loaded over a Bio-Gel P2 column and fractioned with TE buffer (a). (b) Fluorescence spectra of the pooled fractions when excited at 295 nm.

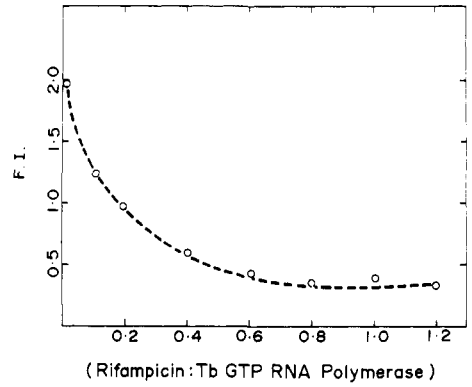

FIGURE 4: Titration of the corrected fluorescence emission of TbGTP–RNA polymerase [3 μM Tb(III), 1 μM GTP, and 10 μM RNA polymerase] with varying concentrations of rifampicin at 545 nm. The excitation wavelength is 295 nm. F.I. indicates the relative fluorescence intensity.

Figure 4. Rifampicin is known to form a 1:1 complex with *E. coli* RNA polymerase (Gurgo, 1980); we observed that the titration of TbGTP–RNA polymerase emission bands with rifampicin was complete when rifampicin:TbGTP–RNA polymerase was around 0.7:1. Deviation from the stoichiometric quenching may arise due to the presence of only 60–70% of active RNA polymerase molecules in any given preparation (see Methods).

Calculation of the Förster's Distance between the Donor (TbGTP–RNA Polymerase) and the Acceptor (Rifampicin) on E. coli RNA Polymerase. Thus, in our case the donor of fluorescence emission was TbGTP complexed with RNA polymerase, not the TbGTP alone, as the enzyme quenched the fluorescence signal of TbGTP further. By use of the K_d values described before, the percent of GTP bound to Tb(III) at 3 μM Tb(III) and 1 μM GTP (see Figure 1b) is calculated to be 80%. Similarly, the percent substrate bound on the enzyme at the saturation concentration of RNA polymerase (10 μM, see Fig. 2) is calculated to be 70%. Therefore, all the relative fluorescences (percent) have been corrected for 100% occupancy of substrate on RNA polymerase. Figure 5 shows the spectral overlap between the donor fluorescence and the acceptor absorption. One may note here that both the emission bands of Tb(III) had finite spectral overlap with rifampicin absorption. Although the emission band at 545 nm had much less overlap in comparison to the 488-nm band, the fluorescence intensity at 545 nm was much higher and thus made the overlap integral value significant. Values of quantum yield, overlap integral, R_0, etc. calculated from eq 1–5 are reported in Table I.

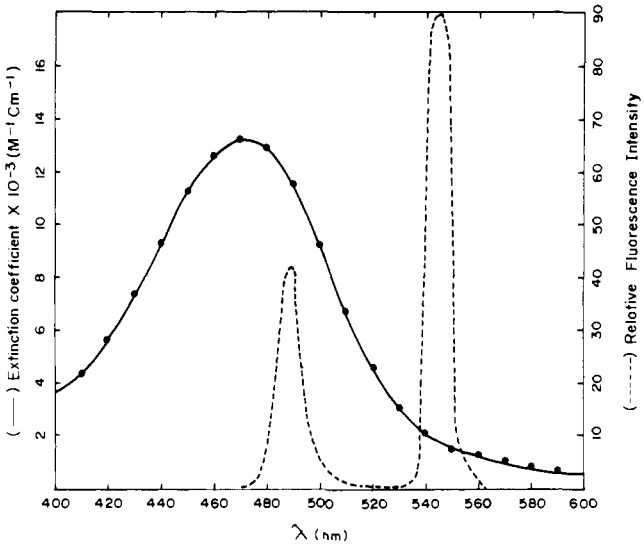

FIGURE 5: Spectral overlap between the absorption spectrum of rifampicin and the corected fluorescence emission spectrum of TbGTP–RNA polymerase when excited at 295 nm. The rifampicin absorption spectrum was recorded in 10 mM Tris-HCl (pH 7.5) and 100 mM KCl buffer.

Discussion

Resonance energy levels of Tb(III) fortuitously overlap with the triplet energy state of GTP ligand, irradiated with ultraviolet light. As a result, an intermolecular energy transfer from the organic ligand to the central metal atom takes place along with radiationless deactivation of the ions themselves. Thus, when excited with appropriate light (λ, 295 nm), the Tb(III)GTP complex shows two emission signals (Horrock & Albin, 1984) generating from the $5D_4 \rightarrow 7F_5$ and $5D_4 \rightarrow 7F_0$ transitions of the metal ions. Tb(III) also shows enhanced emission signals with various proteins provided its binding site

Table II: Various Distances Obtained between Specific Points in *E. coli* RNA Polymerase by Fluorescence Resonance Energy Transfer Study

system	distance (Å)	technique	reference
(i) rifampicin binding site–surface of σ-subunit	min 10	fluorescence energy transfer	Wu et al. (1976)
(ii) intrinsic metal in β-subunit–132-SH of σ-subunit	22	fluorescence energy transfer	Chatterji et al. (1986)
(iii) intrinsic metal in β-subunit–initiation site	17	fluorescence energy transfer	Wu and Tyagi (1987)
(iv) rifampicin binding site–substrate binding site	30	fluorescence energy transfer	this study
(v) intrinsic metal in β-subunit–substrate binding site	4	NMR	Chatterji et al. (1984)
(vi) initiation site–elongation site	6	EPR	Chuknyiski et al. (1986)

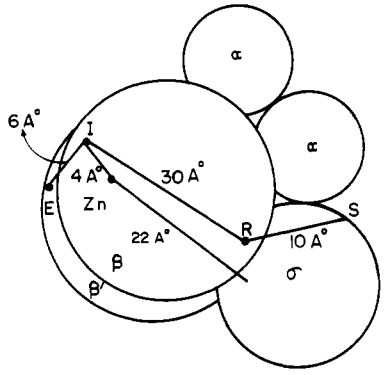

FIGURE 6: Schematic representation of different functional domains in *E. coli* RNA polymerase (please see Table II). I, initiation site; E, elongation site; Zn, intrinsic Zn in β-subunit; S, surface of σ-subunit; R, rifampicin binding site.

is located near one of the aromatic amino acids. This has been efficiently utilized in the past to map the Tb(III) binding site on a protein with respect to other chromophores (Luk, 1971; Berner et al., 1975; Horrocks et al., 1975). However, the present study shows the overlap of the acceptor's absorption with both emission peaks of the donor. Therefore, we have calculated the R_0 values from the quenching of both emission signals of the donor, independently, in the presence of the acceptor. Ideally in such cases both R_0 and r should be the same as they represent the distance between the same two points. Table I reveals that the discrepency in the values of R_0 or r from 488- and 545-nm bands is within 10–20%, which is the experimental and theoretical limit of fluorescence energy transfer.

Meares and Rice (1981) applied their rapid-diffusion limit energy-transfer technique to determine how accessible the rifampicin and the dye cibacron blue were to solvent by using freely diffusing neutral complexes of Tb(III) as luminescent energy donors. It was also mentioned there that the rate of energy transfer from the Tb(III) chelate to the enzyme-bound rifampicin was about half as large as for free rifampicin in solution. For the transfer of energy from the freely diffusing energy donors to acceptors, intermolecular contact between the donor and acceptor is necessary. This mechanism has a very sharp distance dependence; it drops off much more rapidly than the dipole–dipole mechanism. However, in our case the donor molecule is not a neutral complex of Tb(III), and it binds strongly to the protein. Moreover, the direct collision between the donor and the acceptor is not possible as the rifampicin binding domain on RNA polymerase is thought to be within a crevice formed by subunit interaction (Jin & Gross, 1988). Therefore, the diffusion-enhanced energy transfer in our case is extremely unlikely, and we have interpreted our data in terms of a static model. We have mentiond before that Tb(III) has three binding sites on GTP. Therefore, a 3:1 complex between Tb(III) and GTP [3 μM Tb(III) and 1 μM GTP] is best suited for the energy-transfer measurements. Our calculations have shown that about 80% of the GTP is labeled with Tb(III); the free metal ion is nonfluorescent and does not show any appreciable emission in the presence of the enzyme alone. The 70% complexation of the labeled substrate with the enzyme and its incorporation into the template-directed synthesis of RNA again indicate that our data interpretation on the basis of dipole–dipole energy transfer is probably correct. Moreover, the observation that the titration curve of the emission signals of TbGTP–RNA polymerase reached saturation in the presence of a stoichiometric amount of rifampicin support this contention.

The most striking result emerging from the present study is the large distance between the initiator substrate binding site and the rifampicin binding domain on *E. coli* RNA polymerase. It was estimated before by assuming the β-subunit is a spherical protein that its radius is 37 Å (Wu et al., 1976). Therefore, it appears the initiator binding site and rifampicin are located opposite to each other on the same subunit of RNA polymerase. Such a large distance necessarily indicates that rifampicin-induced inhibition of the initiation reaction catalyzed by RNA polymerase may not be competitive.

Earlier fluorescence energy transfer measurements (Wu et al., 1976) showed the rifampicin binding site on *E. coli* RNA polymerase to be within 10 Å of the σ-subunit. One of us has recently measured the distance from the σ-subunit to the intrinsic metal-containing initiation site to be 22 Å (Chatterji et al., 1986). Considering that the metal is located about 4 Å away from the substrate binding site (Chatterji et al., 1984), the distance calculated in this study may be represented on a schematic diagram as shown in Figure 6. Such depiction of *E. coli* RNA polymerase was reported earlier (Hillel & Wu, 1977). Over the years various spectroscopic studies have measured various distances among different functional domains in RNA polymerase (Table II). In Figure 6 we have shown such relationships. It has been recently reported (Heumann et al., 1988) that the center-to-center distance of the subunits β and β′ is greater in the enzyme than in the complex with DNA. In the future, we would like to see whether the geometric relationships among various sites at the β- and β′-subunits also undergo changes in the presence of a promoter DNA fragment.

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APPENDIX

The following FORTRAN program was used to calculate the spectral overlap integral J.

```
      REAL WL (999), EA (999), FI (999)
      Z = 470
      DO 101  I = 1, 41
      WL (I) = Z + 1
 101  Z = WL (I)
      DO 102 I = 1, 41
 102  Read (1, *) EA (I), EI(1)
      OV = 0
      AO = 0
      DO 103  I = 1, 41
```

```
      OV = OV + (FI(I) * EA(I) * (WL(I)**4) * 1E-35)
  103 AV = AV + (FI(I) * 0.0000001)
      AJ = OV/AV
      WRITE (G, *) 'J factor (cm + 3m^-1) = ', AJ
      STOP
```

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