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Specific Activation of Transcription Initiation by the Sequence-Specific DNA-Binding Agents Distamycin A and Netropsin†

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Received July 25, 1986; Revised Manuscript Received October 9, 1986

ABSTRACT: A series of promoters with nine base-pair substitutions in the spacer DNA separating the −10 and −35 regions was used to demonstrate that *Escherichia coli* RNA polymerase is sensitive to events affecting the spacer DNA—a region not directly contacted by the enzyme. The drugs distamycin A and netropsin specifically enhanced the rate of functional complex formation at a promoter bearing a substitution of nonalternating A-T base pairs. The effect is exerted at an early step in the RNA polymerase–promoter interaction. We hypothesize that a drug-induced structural alteration in the spacer DNA occurs, similar to that normally resulting from RNA polymerase binding. These findings are relevant to an understanding of potential mechanisms of transcription activation.

Escherichia coli promoters have two regions of DNA that are contacted by RNA polymerase in a functional or "open" complex. These stretches are referred to as the −10 and −35 regions to indicate their distances upstream from the start site of transcription (Siebenlist et al., 1980; Hawley & McClure, 1983b; von Hippel et al., 1984; McClure et al., 1985). The contacted regions are optimally separated by a spacer of 17 base pairs. We recently conducted an investigation of the kinetics of open complex formation between RNA polymerase and a series of promoter variants bearing block substitutions in their spacer DNA (see Figure 1). We concluded that the sequence of the spacer DNA can influence the kinetics of open complex formation even though the spacer is not contacted by RNA polymerase in the substituted region (Auble et al., 1986). This effect was ascribed to an altered DNA structure adopted by the substituted spacers (Pulleyblank et al., 1985; Kohwi-Shigematsu et al., 1985) that affected the interaction of RNA polymerase with promoter variants S(CC) and S(GG): evidence has accrued from NMR studies (Sarma et al., 1986) that stretches of DNA consisting of nonalternating G-C base pairs are in the A instead of B form.

The availability of promoters with spacers of defined sequence allowed the targeting of the sequence-selective drugs distamycin A, netropsin, and actinomycin D to this region. Since the binding of each drug alters the structure of the DNA (Kopka et al., 1985; Neidle & Abraham, 1985; Klevit et al., 1986), we hoped to thus alter the spacer structure without interfering with the contacts in the −10 and −35 regions. Actinomycin D (see Figure 2) is an antibiotic inhibitor of RNA synthesis that is specific for G-C-containing DNA. It contains two cyclic polypeptides and a phenoxazone ring system that intercalates between adjacent base pairs of the preferred sequence dGpdC; binding causes unwinding of the DNA helix by about 26° per actinomycin D molecule bound (Sobell, 1973; Van Dyke et al., 1982; Lane et al., 1983; Neidle & Abraham, 1985).

Distamycin A and netropsin (Figure 2) are structurally similar, with distamycin A containing three methylpyrrolecarboxamide rings, while netropsin has two such rings and a guanidinium group in place of the third (Zimmer, 1975). They have very similar specificities for A-T-rich regions of double-helical DNA (Van Dyke et al., 1982; Lane et al., 1983) and apparently a preference for regions with stretches of nonalternating A-T base pairs (Wahnert et al., 1975; Zakrzewska et al., 1983). They bind in the minor groove of B-DNA through hydrogen bonding and hydrophobic interactions, covering four to five base pairs (Van Dyke et al., 1982; Kopka et al., 1985). The binding of netropsin widens the minor groove by several angstroms, and the helical axis of the DNA is bent by 8° per molecule bound (Kopka et al., 1985). Distamycin A apparently introduces similar alterations in the DNA structure (Klevit et al., 1986).

We observe promoter-specific effects that are consistent with the known binding specificities and affinities of each of the

†This work was supported by NIH Grant GM31808 to P.L.deH. D.T.A. is a trainee on NIH Grant GM 08056, and J.P.B. acknowledges support from a Joseph E. Silber fellowship from the American Cancer Society, Cuyahoga County Branch.
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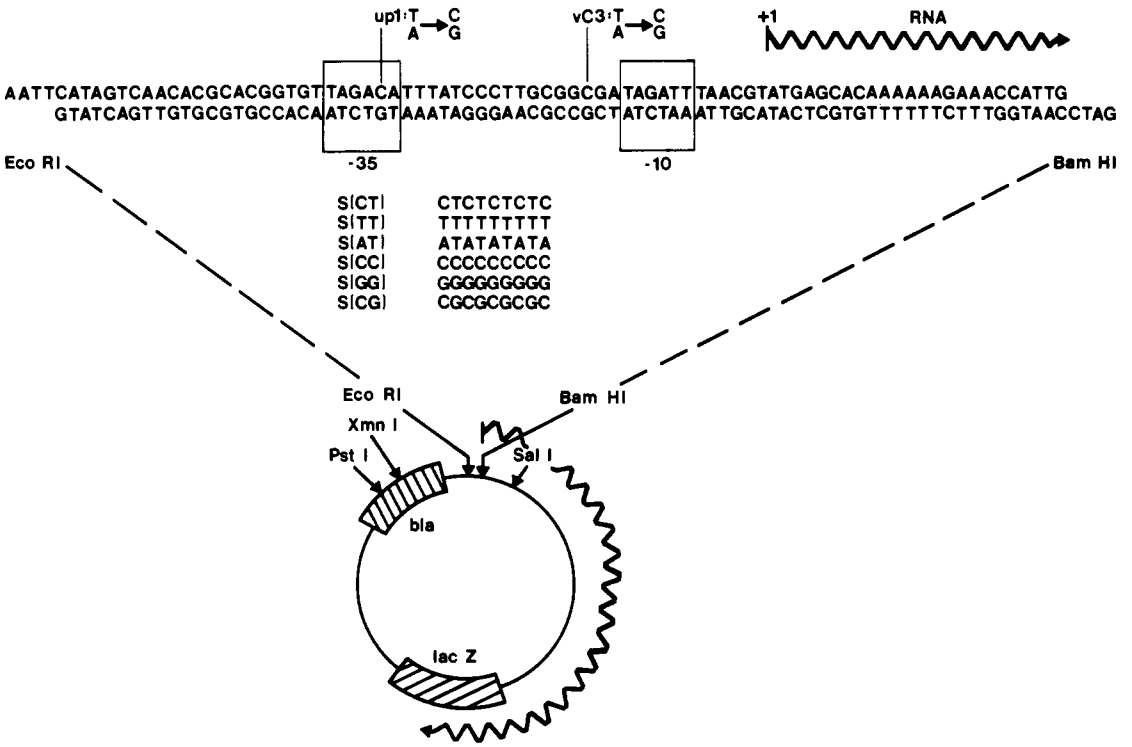

FIGURE 1: Promoter variants used in this study. The promoter with wild-type spacer is shown at the top. Its sequence differs at the indicated positions from that of wild-type P_{RM}. The substituted sequences of the nontranscribed strands of the spacer variants and their positions within the spacer are shown below. All variants were cloned in the vector pRZ5202 as described (Auble et al., 1986).

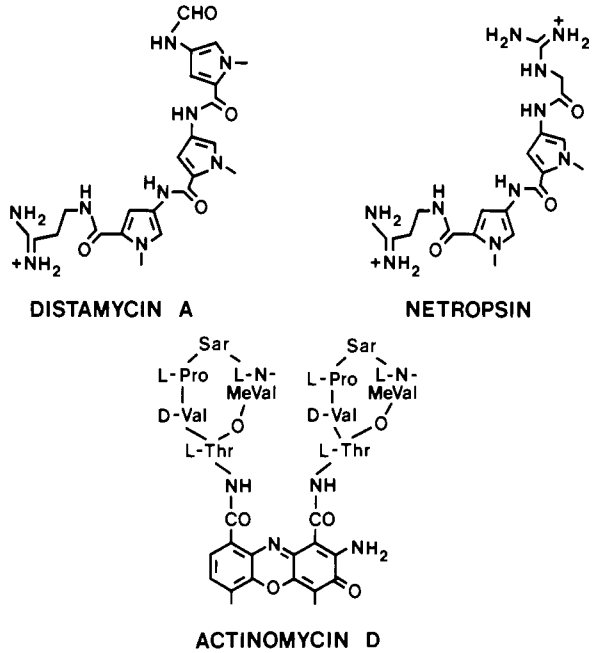

FIGURE 2: Structures of the sequence-specific DNA-binding drugs used in this study, redrawn from Van Dyke et al. (1982) and Neidle and Abraham (1985).

three drugs. It will be argued that selective drug-induced structural alterations are introduced that lead to differential effects on the kinetics of the promoters' interaction with RNA polymerase and on the properties of the resultant open complexes. Actinomycin D causes an inhibition of the RNA polymerase–promoter interactions at all the promoter variants, but at different drug concentrations. A striking preferential acceleration of the rate of open complex formation by low concentrations of distamcyin A (and to a lesser extent netropsin) is observed with S(TT), the promoter containing a spacer of nonalternating A-T base pairs.

Materials and Methods

Chemicals and Enzymes. UTP[1] and UpA were from Sigma. [α-^{32}P]UTP ($\approx$600 Ci/mmol) was from New England Nuclear. Restriction endonucleases were from New England Biolabs. All other chemicals were of reagent grade.

Drugs. Actinomycin D and distamycin A were obtained from Sigma Chemical Co. Netropsin hydrochloride was a gift from Dr. Borders of American Cyanamid, Pearl River, N.Y. All drugs were dissolved in 10 mM Tris-HCl, pH 7.5, containing 10 mM NaCl. Dilutions of the stock were made, and 50-μL aliquots were individually stored at −20 °C and discarded after being thawed. In agreement with others (Dattagupta et al., 1980), we have found that distamycin A solutions lose activity with time even when stored frozen. We have therefore remade our stocks at intervals of approximately 3 weeks.

E. coli RNA polymerase was purified by the method of Burgess and Jendrisak (1975) and then passed over a phosphocellulose column. Enzyme concentrations were calculated with $E^{1\%}_{280nm} = 6.2$. Our preparation of RNA polymerase was found to be 40% active as judged by transcription experiments on T7 DNA (Chamberlin et al., 1979). The reported value for the concentration of RNA polymerase used in each assay has been corrected for the fraction of active enzyme.

DNA. The promoters used in this study are variants of the P_{RM} promoter of phage λ. They were constructed and characterized as previously described (Auble et al., 1986). Abortive initiation experiments (see below) were performed with DNA that was obtained by large-scale plasmid purification as described (Maniatis et al., 1982). Drug titration experiments were performed with plasmid DNA that was preparatively linearized with *Pst*I. Kinetic analyses of functional complex

[1] Abbreviations: UTP, uridine 5′-triphosphate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; BSA, bovine serum albumin.

formation were carried out with the above linearized plasmid DNAs and with promoter-containing 756 base-pair fragments that were obtained by preparative digestion of plasmid DNA with *Xmn*I and *Sal*I, followed by electrophoresis on 8% nondenaturing polyacrylamide gels and electroelution into dialysis bags as described (Maniatis et al., 1982).

Abortive Initiation Assays. The formation of functional ("open") complexes was detected by the abortive initiation assay (McClure, 1980). In the presence of UpA and UTP, RNA polymerase bound at the P_{RM} promoter catalyzes the synthesis of UpApU(Hawley & McClure, 1982). Typical reactions contained standard transcription buffer [30 mM Tris-HCl (pH 8), 0.1 M KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 10 mM DTT, and 50 μg/mL BSA], 1 mM UpA, 45 μM UTP ([α-^{32}P]UTP was added to a specific activity of $\approx$5000 cpm/pmol), and promoter DNA at the indicated concentrations. The synthesis of UpApU was quantitated by chromatography on Whatman 3MM paper as described (McClure, 1980).

Initially, titrations were performed to measure the effects of each of the drugs by the following protocol ("activity assays"). Plasmid DNA (containing a promoter variant) was linearized by incubation with *Pst*I. DNA (1 nM) was preincubated with drug for 10 min in transcription buffer at 37 °C. RNA polymerase was subsequently added to a concentration of 12 nM, and the incubation was continued for 5 min to allow formation of open complexes. Prior kinetic characterization of our promoter variants (Auble et al., 1986; see also Figure 5) indicates that during this incubation about 50% of the promoters should acquire an RNA polymerase bound in an open complex. Thus, this protocol allows us to detect drug-induced activation as well as repression of functional complex formation. Following this incubation, UpA, UTP, and [α-^{32}P]UTP were added to initiate the RNA polymerase catalyzed synthesis of UpApU. After 10 min, the entire reaction volume of 20 μL was spotted onto Whatman 3MM chromatograms for quantitation of the synthesized product. The percent incorporation is determined from the fraction of total counts present in the product. The incorporation of UTP into UpApU in the presence of drug is normalized to the value in its absence. The "activity" of the complexes in this assay depends both on the rate of formation of open complexes and on the rate at which the RNA product is synthesized.

Kinetic experiments ("lag assays") performed in the absence or presence of distamycin A allowed the effects observed in the activity assays to be further analyzed. Reactions were initiated by the addition of RNA polymerase following a 10-min preincubation in the presence or absence (controls) of distamycin A. The reaction volume was typically 55 μL, and 4-μL aliquots were spotted at various times onto Whatman 3 MM chromatograms. All reactions were conducted at 37 $\pm$ 0.2 °C. A computer fit of the data yields τ, the lag time for formation of open complexes (McClure, 1980), and the steady-state rate V_{ss} at which open complexes synthesize the abortive product. In some instances (Figure 4), the reaction was so slow at high drug concentrations that steady state was not reached during the course of the experiment. In these instances the lag times were estimated from the data, neglecting the small effect of the drug on abortive synthesis by already formed open complexes (see Results).

RESULTS

With the titration assay described under Materials and Methods, distamycin A, netropsin, and actinomycin D were each tested for the ability to affect the interaction of RNA

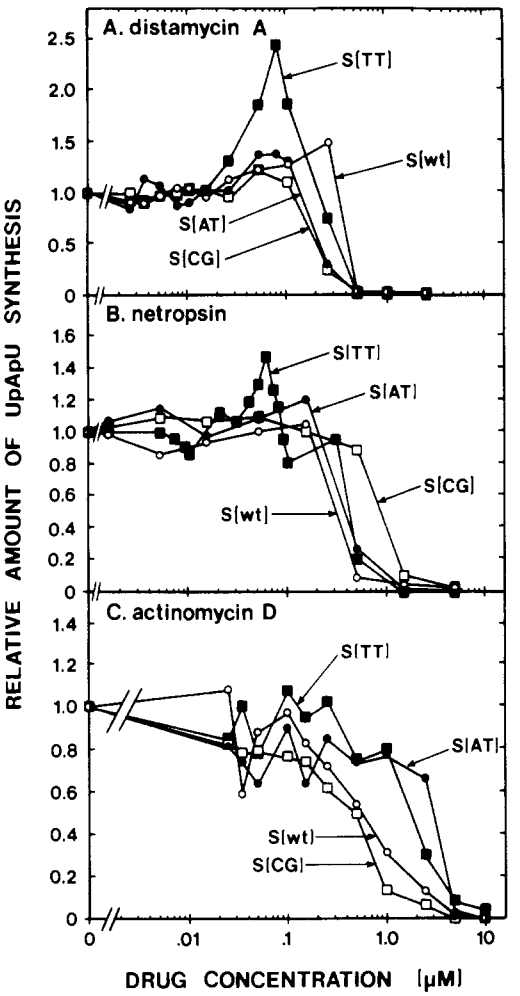

FIGURE 3: Effects of different concentrations of distamycin A, netropsin, and actinomycin D on promoter activity. The amounts of abortive RNA (UpApU) made during a 10-min incubation period after a 5-min preincubation with 12 nM RNA polymerase and plasmid DNA (see text) were measured as a function of the concentrations of the drugs. The data have been normalized to values obtained in the absence of drug. The linearized plasmid DNAs carried the following promoters: (○) S(wt), (■) S(TT), (●) S(AT), and (□) S(CG). For clarity, some curves have been omitted as no preferential inhibitions or stimulations were seen: S(GG) and S(CT) with distamycin A and netropsin; S(CC) and S(CT) with actinomycin D.

polymerase with the promoters shown in Figure 1. The results, obtained with promoters contained on linearized whole plasmid DNA (see Materials and Methods), are shown in Figure 3. The titration curves for distamycin A (Figure 3A) show that, at a drug concentration of 0.08 μM, S(TT)-directed synthesis of UpApU is stimulated to a level more than 2-fold higher than that in the absence of the drug. Much less stimulation is observed at the other promoters. Inhibition to 50% of the no drug value occurs at similar concentrations of the antibiotic (0.1–0.2 μM) for all of the promoters. [The greater apparent resistance to inhibition of S(wt) is a peculiarity of this experiment and does not represent a reproducible effect.] Netropsin (0.06 μM) induces a 1.5-fold stimulation of the abortive reaction with S(TT), while with S(AT) the maximal stimulation is to a relative activity of 1.2 at a somewhat higher drug concentration. Fifty percent inhibition of synthesis occurs at 0.3 μM netropsin, except with S(CG) where a 2-fold higher drug concentration is required. From these experiments, we conclude that there must be at least two types of binding sites for the drug, the higher affinity site resulting in stimulation and the lower affinity site in inhibition. In view of the promoter-specific effects observed, the former site must be on the

Table I: Distamycin A Effects on Rates of Formation of Open Complexes and Their Activity

promoter	k_{obsd}(rel)[a]	V_{ss}(rel)[a]
S(wt)	1.29 ± 0.14 (2)[b]	1.16 ± 0.02 (2)
S(AT)	1.37 ± 0.06 (3)	1.10 ± 0.03 (3)
S(TT)	2.36 ± 0.6 (4)	1.38 ± 0.08 (4)

[a] RNA polymerase concentration for all experiments was 12 nM. Values for both k_{obsd} and V_{ss} obtained in the presence of maximally stimulating distamycin A concentrations (ranging from 0.02 to 0.04 μM in this series of experiments) have been normalized to determinations in the absence of the drug, which were similar for all three promoters: $k_{obsd} = (1.7 \pm 0.4) \times 10^{-3}$ s^{-1} and $V_{ss} = 225 \pm 50$ UpApU per promoter per min, in reasonable agreement with Hawley and McClure (1982). [b] Numbers in parentheses refer to the number of independent determinations.

promoter DNA. We suspect that the latter site is DNA as well but cannot rule out inhibition due to protein-bound drug.

We observe only inhibitory effects with another sequence-specific DNA-binding antibiotic, actinomycin D. The titration in Figure 3C indicates that at an actinomycin D concentration of 0.8 μM the extent of open complex formation is reduced by 50% for the promoter with wild-type spacer [S(wt)] and also for the promoters with G-C-rich spacers [S(CG) and S(CC)]. In contrast, a higher concentration of the drug (3–5 μM) is required to cause a 50% inhibition of the A-T-substituted promoters S(AT) and S(TT). These results are consistent with the known preference of the drug for G-C-containing DNA (Van Dyke et al., 1982; Lane et al., 1983) and demonstrate that the promoter-specific activation of transcription seen with distamycin A and netropsin is dependent on the structure of the DNA-binding ligand. The drug concentrations at which inhibition is observed are in good agreement with estimates of the binding constant for the interaction of actinomycin D with G-C-rich DNA (Sobell, 1973).

In view of the greater stimulation seen with distamycin A, we chose this drug for further studies. The stimulation could be due to an effect of the drug either on the rate of formation of open complexes [with a rate parameter k_{obsd} that is the reciprocal of the experimentally determined lag time τ (see Materials and Methods)] or on the steady-state rate of synthesis of the abortive RNA product by the RNA polymerase–promoter complexes (V_{ss}). To address this question, lag assays (see Materials and Methods) were performed in the presence of distamycin A. In Figure 4, we present a comparison of the rates of open complex formation, k_{obsd}, in the presence of distamycin A for the wild-type promoter [S(wt)] and the two promoters with A-T-rich spacers [S(AT) and S(TT)]. Table I summarizes the data obtained at the distamycin A concentration at which the maximal stimulatory effect was observed.

As indicated in Table I, distamycin A leads to an increase of both k_{obsd} and V_{ss} for all three promoters, but in each case the increase in k_{obsd} is 2–3-fold greater than that in V_{ss}. In agreement with the titration results presented in Figure 3A, the drug-induced increase in the values of k_{obsd} and V_{ss} are 2–3-fold greater for S(TT) than for the other two promoters. The effects on k_{obsd} and V_{ss} are independent: at distamycin A concentrations sufficient to decrease k_{obsd} to less than half its maximal value, V_{ss} is still close to maximal (data not shown).

We also determined the effect of the three drugs on preformed open complexes. No increase of V_{ss} was seen in the presence of a low concentration (0.05 μM) of distamycin A that was stimulatory in the activity assay (Figure 3). Additionally, concentrations of any of the three drugs that were completely inhibitory in the activity assay were found to have

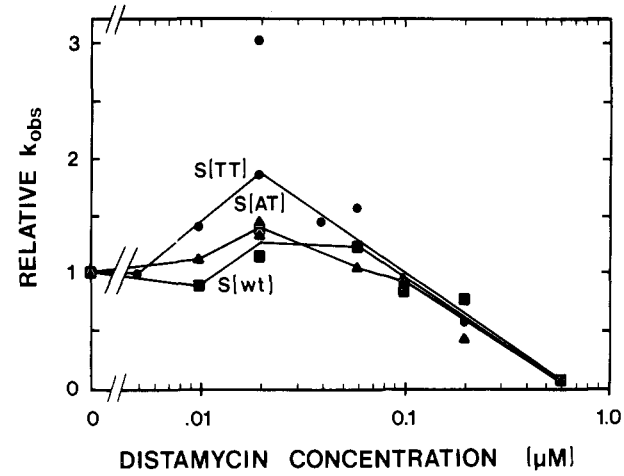

FIGURE 4: Distamycin A concentration dependence of the rate of open complex formation with three promoter variants contained on restriction fragments: (■) S(wt), (●) S(TT), and (▲) S(AT). The values for k_{obsd} obtained in the presence of distamycin A have been normalized to measurements in the absence of the drug [$k_{obsd} = (1.7 \pm 0.4) \times 10^{-3}$ s^{-1}, averaged for the three promoters]. The reactions contained 12 nM RNA polymerase; rates were determined as described in the text.

no effect on the synthesis of UpApU by preexisting complexes (data not shown). Thus, access of the drugs to their binding sites is obstructed in the open complex.

The maximal effect of distamycin A in the lag assays (Figure 4) occurs at a drug concentration that is almost 4-fold lower than the drug concentration required for an optimal effect in the activity assays (Figure 3). The range of distamycin A concentrations for an effect on k_{obsd} is found to be 0.02–0.04 μM, while in the activity assay it is 0.05–0.1 μM. A major reason for this discrepancy is the fact that the lag assays are performed with 0.2 nM *Xmn*I–*Sal*I fragment, while the activity assay reactions contain 1 nM plasmid. This represents a large difference in total DNA content, necessitating the addition of more drug in order to compensate for the additional amount bound to the DNA. Indeed, control titrations with the activity assay indicate that optimal stimulation requires a 2-fold lower drug concentration when 1 nM DNA fragment is used rather than intact plasmid DNA (data not shown).

Having determined that the main effect of distamycin A is the acceleration of the formation of open complexes, we wished to further dissect this effect into the binding of RNA polymerase to promoters and the subsequent formation of "open" or active complexes. The following scheme has been useful for the representation of the sequence of events leading to the formation of RNA polymerase–promoter open complexes (McClure, 1986):

$$R + P \underset{}{\overset{\text{binding}}{\rightleftharpoons}} RP_c \overset{\text{isomerization}}{\rightleftharpoons} RP_o$$

Here RNA polymerase (R) and promoter (P) combine in rapid equilibrium (association constant K_B) to form an inactive or closed complex RP_c that isomerizes (rate constant k_f) to the functional or open complex RP_o. The values of the parameters K_B and k_f can be determined from the dependence of the measured τ values on the concentration of RNA polymerase. A plot of τ vs. the reciprocal of the RNA polymerase concentration (a "τ plot") is linear, with a y intercept of k_f^{-1} and a slope of $(K_B k_f)^{-1}$. Thus, it is possible to determine whether distamycin A affects the initial binding of RNA polymerase to the promoter, the subsequent isomerization step, or both. The above analysis is a reasonable first approach to

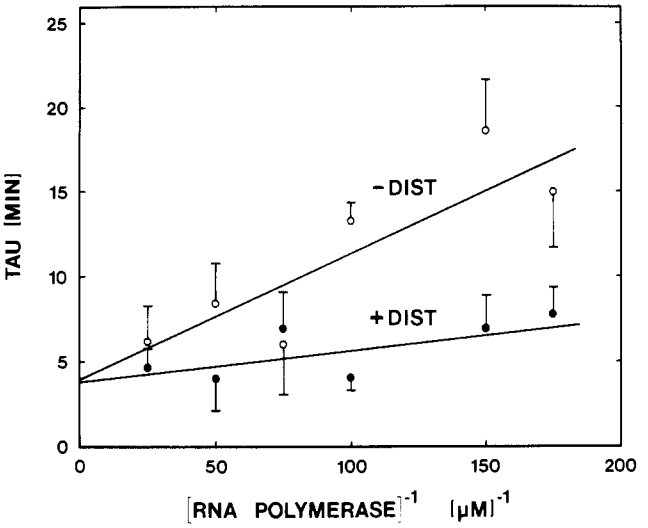

FIGURE 5: Dependence of the rate of open complex formation at S(TT) (see Figure 1) on the RNA polymerase concentration in the absence (○) and the presence (●) of 0.05 μM distamycin A. The promoter-bearing *Xmn*I–*Sal*I restriction fragment was present at 0.2 nM. The reciprocal of the rate constant (i.e., the lag time τ) is plotted vs. the reciprocal of the RNA polymerase concentration. Note the clearly reduced RNA polymerase concentration dependence in the presence of distamycin A.

the interpretation of the kinetic data (McClure, 1980; Hawley & McClure, 1982, 1983a; Shih & Gussin, 1983). It should be kept in mind, however, that the representation with just one intermediate on the path to open complex formation is a simplification of the overall process (Hawley & McClure, 1982; Roe et al., 1985). While the mechanistic interpretation of the parameters obtained from τ plots may depend on the details of the interaction, K_B will still be representative of the reversible, concentration-dependent steps and k_f of the subsequent, essentially irreversible process.

Such an analysis is presented in Figure 5 for promoter S(TT) contained on the same 756 base-pair *Xmn*I–*Sal*I fragment that had been used previously for kinetic studies on these promoter variants (Auble et al, 1986). A τ plot obtained in the presence of 0.05 μM distamycin A is compared to one determined in the absence of the drug. It is readily apparent that the major difference between the two curves is the much shallower slope obtained in the presence of distamycin A. The two lines, however, have similar intercepts. This indicates that in the presence of the drug the binding of RNA polymerase to form the closed complex is increased, without the rate of isomerization to open complexes being significantly affected. The results of analogous experiments with other promoters, contained on restriction fragments as well as on plasmid DNA, are compiled in Table II. It can be seen from Table II that for S(TT) distamycin A leads to an increase in K_B of a factor of 4 without affecting k_f, while for S(wt) and S(AT) the effects of the drug are insignificant compared to the experimental uncertainty in the data.

Table II: Distamycin A Effects on Kinetics of Open Complex Formation: τ Plots and Kinetic Parameters

	without distamycin A		with distamycin A[a]	
promoter	K_B (μM^{-1})	k_f (×10^3 s^{-1})	K_B (μM^{-1})	k_f (×10^3 s^{-1})
S(wt)	78 ± 12[b]	3.1 ± 0.3	78 ± 28	4.1 ± 1.0
S(AT)	73 ± 21	6.1 ± 1.3	59 ± 1	5.1 ± 1.4
S(TT)	58 ± 6	5.0 ± 0.8	>250[c]	4.3 ± 0.1

[a] The data are averages of two determinations, one with the promoter on whole plasmid DNA and the other with the *Xmn*I–*Sal*I restriction fragment. The amount of distamycin added was 0.08 μM for the former and 0.05 μM for the latter case (see text). [b] The data are presented as average ± half of spread of the individual determinations; the computer fits to the τ plots yield errors of about 50% in K_B and 30% in k_f. [c] Lower limit estimated on the basis of the range of RNA polymerase concentrations used and the accuracy of the data.

Discussion

Our results show clear effects of distamycin A, netropsin, and actinomysin D on the promoter variants. We will first discuss the implications of these results for our further understanding of the binding specificities of these drugs and then consider their implications for our understanding of promoter function and activation.

While the results confirm that distamycin A and netropsin show similar binding behavior, they also underscore the fact that these drugs do not behave identically: the stimulatory effect observed with distamycin A is significantly more pronounced than the effect observed with netropsin. In view of the otherwise identical structure of the two compounds, this difference must be due to the fact that distamycin A has a third methylpyrrolecarboxamde ring, while at the position netropsin has a guanidinium group. Both drugs distinguish between the A-T-containing promoters S(TT) and S(AT), since they activate S(TT) to a greater extent than S(AT). While a priori this difference needs not translate into a binding preference for poly(dA)·poly(dT) over poly(dA-dT)·poly(dA-dT), there are reports that describe just such a preference (Wahnert et al., 1975). An alternative explanation is that the drugs introduce different structural changes into the alternating and homopolymer stretches of DNA.

Promoters with A-T-rich spacers [S(TT) and S(AT)] are more refractory to inhibition by actinomycin D than the other variants. Presumably, this difference reflects the greater binding affinity of actinomycin D for G-C-containing DNA. There are several possible ways in which an actinomycin D molecule bound to the spacer DNA could cause inhibition of open complex formation. The drug-induced unwinding of the spacer might unfavorably alter the relative positioning of the −10 and −35 regions so that contacts between the binding sites and RNA polymerase can no longer be made. Alternatively, the bulky antibiotic could—while bound to the spacer—sterically interfere with the binding of RNA polymerase. Presently, we cannot distinguish between these possibilities. In view of the small size of the actinomycin D binding site on DNA [five to six base pairs (Van Dyke & Dervan, 1983)], it seems improbable that spacer-bound drug molecules could extend as far as the −10 or −35 regions and block RNA polymerase contact sites.

The activation of transcription initiation by distamycin A and netropsin can be explained by one of two general models, initially proposed to account for the stimulation of transcription initiation at the *lac* promoter by the CRP protein. In the first, activation occurs as a consequence of a direct physical interaction between bound activator and RNA polymerase (Gilbert, 1976). The wild-type promoter used in this study is activated by such a mechanism involving the λ repressor (see below). However, we favor a second model, namely, that the stimulatory effect is due to a change in the structure of the promoter DNA (Dickson et al., 1975) induced by the binding of the drugs.

A facilitation of polymerase binding through physical contact between distamycin A (or netropsin) and promoter-bound RNA polymerase (as in the first model above) is difficult to envision, as this would involve entirely fortuitous favorable contacts between these molecules that are constrained in their relative orientations by being bound to the promoter. Such an interaction is also unlikely in view of the small size of the

antibiotics. Alternatively, the stretch of A-T base pairs in the spacer of S(TT) may provide a nonfunctional binding site for RNA polymerase that prevents the formation of an open complex. The binding of distamycin A or netropsin at the spacer would then prevent the binding of RNA polymerase in the nonfunctional mode and, hence, allow more rapid formation of open complexes. This possibility is unlikely as our characterization of the series of promoters (Auble et al., 1986) has not given any indication that open complex formation on S(TT) is slower than on the other promoters.

We do not yet know the number or configuration of drug molecules on S(TT). As the site sizes of distamycin A and netropsin are four and five base pairs, respectively (Van Dyke et al., 1982; Van Dyke and Dervan, 1983), and S(TT) has 12 uninterrupted A-T base pairs in the spacer DNA, a reasonable assumption is that two drug molecules are simultaneously bound to the spacer. We hypothesize that the antibiotics exert their stimulatory effect by introducing an altered relative spatial orientation of the −10 and the −35 regions that facilitates the binding of RNA polymerase. As a result, less binding energy is expended by the polymerase to bring about this structural change, and a higher binding constant K_B is observed for the formation of the closed complex. DNase I footprinting experiments on open complexes support the notion that RNA polymerase binding has an effect on the structure of the spacer DNA: hypersensitive sites are usually observed in this region (Schmitz & Galas, 1979; Russell & Bennett, 1981).

Two potentialy relevant effects of distamycin A and netropsin on DNA structure will be considered. Analysis of the structure of crystals of netropsin-DNA complexes has shown that the drug bends the helical axis of DNA by 8° (Kopka et al., 1985). As solution NMR studies indicate that the distamycin A–DNA complex is very similar (Klevit et al., 1986), it is likely that this drug would introduce a bend in the DNA as well. Additionally, it has been reported from solution studies that bound netropsin winds the DNA helix by about 10° (Snounou & Malcolm, 1983). The effect of two molecules simultaneously bound to the spacer DNA could be cumulative, leading to a larger effect on the relative orientation of the −10 and −35 regions.

Studies on the activation of transcription at the *prmup-1* promoter of phage λ [equivalent to our S(wt)] by the λ repressor indicate that in the presence of repressor the isomerization rate, k_f, is increased (Hawley & McClure, 1982; Shih & Gussin, 1983) rather than K_B as is the case here. Another difference with the results described here is that the activation is the result of direct protein–protein contacts between repressor and RNA polymerase (Hawley & McClure, 1983a; Hochshild & Ptashne, 1983). Recent experiments have provided strong evidence that the activation of transcription at several other promoters, both prokaryotic (Garner & Revzin, 1981; Spassky et al., 1984) and eukaryotic (Brent & Ptashne, 1985; Takahashi et al., 1986), is due to favorable contacts between protein factors and RNA polymerase bound to promoter DNA. In view of these findings, the possibility that DNA-mediated effects play a role in the regulation of gene expression has been largely ignored.

We previously reported our findings indicating that the intrinsic structure of the DNA of a promoter can affect its interaction with RNA polymerase (Auble et al., 1986). In this paper, we have presented evidence that small, sequence-selective drugs that have the potential to locally alter DNA structure can lead to specific activation of transcription. These findings strengthen the case for DNA-mediated effects as a potential mechanism whereby modulation of transcription initiation can occur.

ACKNOWLEDGMENT

We thank Dr. D. Borders (Lederle Laboratories) for a generous gift of netropsin.

Registry No. Distamycin A, 636-47-5; netropsin, 1438-30-8; actinomycin D, 50-76-0; RNA polymerase, 9014-24-8.

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Mammalian α-Polymerase: Cloning of Partial Complementary DNA and Immunobinding of Catalytic Subunit in Crude Homogenate Protein Blots†

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Received July 16, 1986; Revised Manuscript Received October 8, 1986

ABSTRACT: A new polyclonal antibody against the α-polymerase catalytic polypeptide was prepared by using homogeneous HeLa cell α-polymerase. The antibody neutralized α-polymerase activity and was strong and specific for the α-polymerase catalytic polypeptide (M_r 183 000) in Western blot analysis of crude extracts of HeLa cells. The antibody was used to screen a cDNA library of newborn rat brain poly(A+) RNA in λgt11. A positive phage was identified and plaque purified. This phage, designated λpolα1.2, also was found to be positive with an antibody against *Drosophila* α-polymerase. The insert in λpolα1.2 (1183 base pairs) contained a poly(A) sequence at the 3′ terminus and a short in-phase open reading frame at the 5′ terminus. A synthetic oligopeptide (eight amino acids) corresponding to the open reading frame was used to raise antiserum in rabbits. Antibody affinity purified from this serum was found to be immunoreactive against purified α-polymerase by enzyme-linked immunosorbent assay and was capable of immunoprecipitating α-polymerase. This indicated the λpolα1.2 insert encoded an α-polymerase epitope and suggested that the cDNA corresponded to an α-polymerase mRNA. This was confirmed in hybrid selection experiments using pUC9 containing the cDNA insert and poly(A+) RNA from newborn rat brain; the insert hybridized to mRNA capable of encoding α-polymerase catalytic polypeptides. Northern blot analysis of rat brain poly(A+) RNA revealed that this mRNA is ~5.4 kilobases.

Evidence from inhibitor studies points to a synthetic role of α-polymerase in genomic DNA replication in mammalian cells (Fry, 1982). Activity of α-polymerase is induced as quiescent cells are stimulated to become mitotically active (Fry, 1982; Bollum, 1975; Chang & Bollum, 1973), and conversely, levels of the enzyme decline as actively growing cells become mitotically quiescent (Chang et al., 1973). The mechanisms by which α-polymerase levels are regulated are not understood and are a subject of research interest in these and several other laboratories. Our current approach to this problem is to develop molecular probes for the α-polymerase mRNA and gene. Another important intermediate step has been the definition of α-polymerase catalytic subunits in their native undegraded form in a growing mammalian cell. Karawya et al. (1984) used monoclonal antibody immunoprecipitation and subsequent enzymatic activity analysis to identify a 180-190-kDa[1] protein as the predominant α-polymerase catalytic subunit in cultured monkey cells. Similar results were obtained by Masaki et al. (1984) with calf thymus. For several reasons, we consider immunoblotting analysis of a crude cell extract a much more reliable method than immunoprecipitation for identification of a native polypeptide. Hence, attempts to confirm the immunoprecipitation results were conducted by probing protein blots with monoclonal antibodies to α-polymerases of calf thymus (Swack et al., 1985) or human KB cell (Tanaka et al., 1982). These experiments were not successful. Therefore, in the present work we developed a new specific polyclonal antibody to α-polymerase that gives strong signals

† This work was supported in part by NIH Grants P30 128-708 and CA 15187 to Earl Baril (Worcester Foundation for Experimental Biology); J.K.V. is a Fellow of the Leukemia Society of America.
‡ National Institutes of Health.
§ Worcester Foundation for Experimental Biology.
∥ The Food and Drug Administration.

[1] Abbreviations: kDa, kilodalton(s); SDS, sodium dodecyl sulfate; IgG, immunoglobulin G; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; TCA, trichloroacetic acid; kb, kilobase(s); MOPS, 3-(*N*-morpholino)propanesulfonic acid; PBS, phosphate-buffered saline; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); SSC, standard saline citrate; BSA, bovine serum albumin; CSA, chicken serum albumin; ADH, adipic dihydrazide; ELISA, enzyme-linked immunosorbent assay; bp, base pair(s).