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Selective Repression of Transcription by Base Sequence Specific Synthetic Polymers†

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ABSTRACT: We report the effect of novel synthetic polymers on deoxyribonucleic acid (DNA) directed ribonucleic acid (RNA) synthesis in vitro. Polymers contained base-selective monomers, including a GC-specific phenazine derivative and an AT-specific triphenylmethane dye. Radical chain polymerization was carried out in aqueous solution by using monomers bound to a template DNA, which was obtained from either λ or T7 bacteriophage. Polymers were isolated and reannealed with DNA samples, including competitive mixtures of T7 and λ DNAs. We measured transcription from DNA–polymer complexes by using *Escherichia coli* RNA polymerase and determined not only the reduction in total transcription levels but also the relative inhibition of λ- or T7-specific transcription by using a hybridization assay. The results show that micromolar concentrations of individual dyes are sufficient to cause substantial inhibition of transcription when the dyes are incorporated into polymers. More significantly, a number of the polymers inhibited more strongly transcription from the DNA which had served as template for polymer synthesis than from the DNA present as competitor in the annealing process. We conclude that template synthesis of DNA-binding polymers can lead to preferential inhibition of function of the original template. The apparent relative affinity of polymer for competing DNAs can be altered by at least an order of magnitude depending on which DNA was used as the synthesis template. The results offer a new approach to improving the specificity of DNA-binding drugs.

Control of gene expression at the DNA level is a primary mechanism for natural regulation of cellular activity. Thorough studies of several procaryotic operons, such as that controlling lactose metabolism in *Escherichia coli* (Bourgeois & Pfahl, 1976), have revealed a general mechanism for the prevention of DNA expression. A protein repressor, possessing a high preferential affinity for an operator base sequence which is located near a promoter site, binds to the operator and inhibits transcription of adjacent genes.

Alteration of the rate of mRNA synthesis by this general mechanism suggests a novel possibility for therapeutic intervention in cellular activity. It is already known that drugs such as actinomycin and daunomycin bind tightly to DNA and may act intracellularly by binding to DNA and inhibiting mRNA synthesis (Gabbay et al., 1976; Kersten et al., 1960; Goldberg et al., 1962; Hurwitz et al., 1962; Hyman & Davidson, 1970). However, these agents are vastly less specific than natural repressors in their DNA affinity. The consequence is high toxicity because of generalized action at sites other than the actual therapeutic target.

Artificial repressors of higher specificity potentially could decrease toxicity, but their synthesis is an imposing task. Although considerable progress has been made in determining which DNA bases participate in specific DNA–repressor complexes (Gilbert & Maxam, 1973; Goeddel et al., 1977), too little is known about the origin of the specific interactions between a DNA sequence and its repressor to justify an attempt to design and synthesize a protein repressor for a chosen DNA target. Hence, some alternative approach is necessary, preferably one based on interactions for which local specificity can be experimentally established.

One such approach would be to prepare polymeric ligands by using as components DNA-binding drugs whose affinity depends on the base composition or sequence of the DNA sample. We have pursued this avenue jointly with the laboratory of W. Müller and H. Bünemann, in an effort that grew out of our shared interest in base-selective DNA-binding drugs (Müller & Crothers, 1968, 1975; Crothers, 1968; Müller et al., 1973, 1975; Müller & Gautier, 1975; Davanloo & Crothers, 1976a,b). If a mixture of base-specific monomers were used for polymerization, one might expect the DNA-binding affinity of the polymer to depend on the match between the DNA base sequence and the sequence of drug monomers in the synthetic polymer.

A central feature of the method is utilization of the target DNA as a template for synthesis of the complementary polymer. H. Bünemann, N. Dattagupta, and W. Müller (unpublished experiments) have found that a mixture of acryl derivatives which includes base-specific dyes can be polymerized while bound to native DNA in aqueous solution. The

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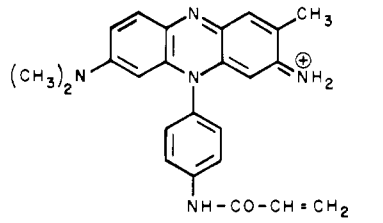
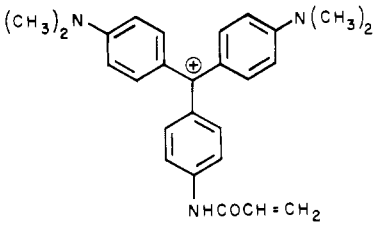
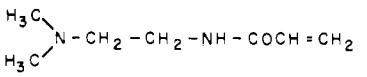


(APNR)



(AMG)



(MA)

FIGURE 1: Chemical structures of the three most commonly used monomers. (Top) Acryl phenyl neutral red (APNR); (middle) acryl malachite green (AMG); (bottom) methylacrylamine (MA).

resulting polymers can be separated from the template DNA in concentrated CsCl solution and assayed for DNA-binding specificity.

Efforts in our own laboratory to date have focused on development of techniques for the in vitro assay of the biological activity and specificity of dye polymers that form soluble DNA complexes. In this paper, we report experimental studies of the inhibition of RNA synthesis by drug polymers which we prepared by utilizing either T7 or λ bacteriophage DNA as template. The results permit three main conclusions. (1) Incorporation of relatively inactive drugs into DNA-binding polymers greatly enhances their ability to inhibit DNA function. Only micromolar concentrations of individual drugs incorporated into polymers are required to give high levels of inhibition of RNA synthesis. (2) Polymers bind to and inhibit transcription of both template and nontemplate DNAs. For example, polymer synthesized on T7 DNA strongly inhibits mRNA synthesis on λ DNA. (3) Polymers can be produced which preferentially inhibit transcription from their template DNA, provided that the polymer is annealed with a competitive mixture of template and nontemplate DNAs. Hence, these polymers share with repressors the properties of generalized DNA binding and selectivity for particular base sequences, albeit at a level well below the selectivity observed for natural repressors.

Materials and Methods

Monomers. Synthesis of the acryl derivatives of the GC-specific intercalator phenyl neutral red, designated acryl phenyl neutral red or APNR, and of AT-specific malachite green, designated acryl malachite green or AMG, was carried out by H. Bünemann, N. Dattagupta, and W. Müller (unpublished experiments). We are grateful to Dr. H. Bünemann for providing samples of these materials. Chemical structures of the specific monomers and the relatively nonspecific methylacrylamine, MA, are shown in Figure 1. In addition to the monomers shown in Figure 1, we synthesized polymers by utilizing vinylimidazole (VI) and the quaternary derivative (QMA) of MA (Figure 1).

Bacteriophage. T7 wild type bacteriophage was grown and purified according to Kosturko et al. (1979). Two strains of λ bacteriophage were used in polymer preparation and assays: λc71 DNA preparation is described in Carter & Radding (1971) and λcI857S7 DNA was generously provided by J. Auerbach.

DNA samples for drug polymerization were prepared by phenol extraction of T7 or λ bacteriophage in TES buffer (10 mM Tris, 1 mM EDTA, and 100 mM NaCl, pH 7.4). Residual phenol was extracted with ether, and the ether was removed by bubbling N_2 through the solution. The resulting solutions were sonicated for 3 min at full power on a Branson Model S125 sonicator, yielding a DNA of molecular weight of about $(1-3) \times 10^6$, as judged by sucrose density gradient sedimentation. Following sonication, samples were dialyzed against BPE buffer (8 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM Na_2EDTA).

Polymerization. Polymerization was carried out by using acrylamide derivatives dissolved in BPE buffer, 25 °C, in the presence of DNA. A typical reaction volume was 600 μL, containing DNA at 0.45 mM base pairs or ~300 μg/mL. The concentration of monomers depended on the desired input ratios; typical values were 0.07 mM APNR, 0.04 mM AMG, and 23 mM MA. One microliter of a 1:10 dilution of *N,N,N',N'*-tetraethylethylenediamine was added, and the reaction was initiated by adding 25 μL of a 50 mg/mL solution of ammonium persulfate in BPE buffer and then bubbling nitrogen gas through the solution. After ~5–15 min the solution viscosity increased greatly and the sample was centrifuged at 5000 rpm to pellet the DNA–polymer complex.

The supernatant was analyzed for the amount of unpolymerized monomers, using visible absorbance spectroscopy in the presence of 2.5% (by weight) sodium dodecyl sulfate ($NaDodSO_4$) to determine the amount of each dye. The total of double bonds in the supernatant was determined by titration with $KMnO_4$ in aqueous solution (King, 1936). To 2.4 mL of $KMnO_4$ solution in water (A_{545} = 1.0 for a 1-cm path), we added x mL of solution containing the double bond and $(0.1 - x)$ mL of H_2O was added. The solution was shaken well, and after exactly 3 min the difference in absorbance at 545 nm was measured against a blank which contained $x = 0$ double-bond solution. Initially, acrylamide (Eastman Organic) and triethylamine (anhydrous, Eastman Organic) in a 1:1 molar ratio were used as standards. Once the concentration of the stock solution of MA was accurately determined, this was used as a standard for subsequent measurements. The amount of unpolymerized amine was calculated from the total measured double bonds by subtracting the spectroscopically measured concentration of unpolymerized dyes.

Purification of Polymers. A solution of 35% (w/w) CsCl was added to the low-speed pellet containing the DNA–polymer complex. After shaking to dissolve the pellet, we centrifuged the solution at 15 °C for 72 h in a SW 50L rotor at 30000 rpm in a Beckman L2-65B preparative ultracentrifuge. The portion of the polymer freed of DNA forms a sharp band at the top of the gradient whereas the DNA, with 10–15% of the polymer attached, sediments to the bottom. Recovery of the total colored monomers as polymer and unpolymerized material was ~80–90%; only 5–10% of the colored material was unpolymerized. Lack of quantitative recovery was found to be relatively more pronounced for malachite green, possibly due to radical chain transfer and subsequent destruction of the chromophore.

We found it essential that every precaution be taken to avoid detergents in polymer preparation and purification since detergents can prevent DNA–polymer complex formation in the assay. Our precautions normally included acid-washing all

glassware, avoiding all plastic ware, and using only doubly distilled water in the preparation of all solutions. Moreover, some commercial preparations of CsCl, even though sold as "density gradient quality" and further purified by charcoal absorption, still contain appreciable quantities of detergent. Following purification, polymers were stored as a CsCl gradient fraction at 4 °C and appear to be stable over a period of at least a year.

Assays of Polymer Sequence Specificity. (a) Reassociation of Polymers with DNA Mixtures. Purified polymer, prepared on either a T7 or λ DNA template, was added to a mixture of the two DNAs each at a concentration of 6 μg/mL in 4 M NaCl and 0.01 M Tris, pH 8.0. The DNA used in assaying polymer specificity was freshly prepared by gentle phenol extraction of intact phage to assure a high molecular weight.

Polymer/DNA samples and a control sample containing the DNA mixture were then dialyzed at 4 °C batchwise first into 4 M NaCl and 0.01 M Tris, pH 8.0, and then into 1.0 M $NaClO_4$, 0.5 M NaCl, and 0.01 M Tris, pH 8.0, followed by gradient dialysis vs. 0.5 M NaCl and 0.01 M Tris, pH 8.0, and finally a last batchwise dialysis vs. 0.5 M KCl and 0.01 M Tris, pH 8.0. The gradient dialysis proceeded slowly, usually over a period of 24–48 h to allow extensive time for annealing the polymer–DNA complex. Following the last batch dialysis, polymer/DNA samples were transferred to test tubes and stored at 4 °C until the samples were assayed for sequence specificity.

(b) In Vitro Transcription Assay. A 10-μL portion of each polymer/DNA sample chilled to 0 °C was added to a small polystyrene tube (Falcon) containing 65 μL of a cocktail, also at 0 °C. After the addition of the polymer/DNA sample, each tube contained 44 mM Tris, pH 7.9, 1.3 mM dithiothreitol, 5.03 mM $MgCl_2$, 13.3 μM EDTA, 67 mM KCl, 2 μg of bovine serum albumin, 0.4 mM ATP, CTP, and GTP, 4 μM UTP, 1 μCi of [^{3}H]UTP, and 1.0 unit of *E. coli* K12 RNA polymerase (Enzo Biochem, Inc., or Boehringer Mannheim). The mixture was immediately transferred to a 37 °C water bath for 10 min, at which time a 10-μL portion was precipitated in a test tube containing 3 mL of 3.5% $HClO_4$ and 0.1 M sodium pyrophosphate, 0 °C, and kept at that temperature for at least 30 min until it was further processed.

At 10 min, 10 μL of DNase I (RNase-free, Worthington Biochemical Corp.), 5 mg/mL in TES buffer, was added to the remainder of the mixture in the test tube and the solution was incubated for an additional 15 min at 37 °C. At this time, 25 min after the initiation of the assay, the mixture was transferred to an ice bath and immediately 2 mL of sterile 2 × SSC (0.3 M NaCl and 0.03 M sodium citrate) was added to each tube, along with 10 μg of yeast RNA to serve as cold carrier during the extraction process.

Each sample was then phenol extracted and washed twice with prewashed ethyl ether to remove dissolved phenol. Nitrogen was bubbled through each sample to remove ether, and finally the mixture was filtered through a nitrocellulose filter (Millex, 0.22 μm, Millipore Corp.) to remove the dye polymers, which can inhibit RNA–DNA hybridization. The filtrate was placed in a vial containing three nitrocellulose membranes (Schleier and Schuell, B-6), one having T7 single-stranded DNA affixed (Gillespie & Spiegelman, 1965), one with single-stranded λ DNA affixed, and one blank for the hybridization process. Initial experiments in which the single-stranded DNA fixed to the membranes was labeled with ^{32}P demonstrated the reproducibility of this preparation.

The hybridization reaction was carried out by incubating the vial at 55 °C for 18 h, at which time 50 μL of RNase A (aqueous solution, 1 mg/mL, made DNase-free by boiling for 10 min followed by rapid cooling) was added to each vial. The vials were then incubated for 30 min at 37 °C with gentle agitation. Finally, the filters were separated and washed 4 times on each side with 3 × SSC, transferred individually to scintillation vials, and then dissolved in Aquasol and counted in a Packard Tri-carb scintillation counter.

Table I: Character and Composition of Dye Polymers

polymer no.	rel molar ratios of AMG/APNR/MA used for polymerization	rel molar ratios of AMG/APNR/MA incorporated in polymer	template DNA
N4	0.41:1:700	0.4:1:100	T7
J1	0.31:1:700	0.30:1:130	T7
Ag1	0.56:1:300	0.5:1:170	T7
D2	0.4:1:600	0.4:1:130	λ
D3	0.4:1:300	0.4:1:80	λ
M3	0.35:1:250	0.3:1:21[a]	λ
Ag2	0.56:1:300	0.4:1:150	λ
F3	0.24:35.7:11.4[b]	1:100 ± 30[c]	T7

[a] $r_{APNR} = 0.15$ (drug molecules per base pair) during polymerization; in all other cases, $r_{APNR} = 0.08 \pm 0.02$. [b] APNR/VI/QMA. [c] APNR/total double bonds.

The counts hybridized to the T7 filter or to the λ filter, after subtracting from each the counts absorbed to the blank filter, reflect the relative amount of each species of RNA transcribed in the DNA mixture. For a polymer/DNA sample, specificity can be evidenced as a decrease in the counts hybridized on one of the two phage DNA filters. However, because the amount of RNA hybridized to any filter reflects the efficiency of extraction and the efficiency of hybridization as well as the amount of transcription in that sample, monitoring a simple decrease in the counts on any one species filter is not the most reliable way to ascertain specificity. A more dependable parameter is the ratio of counts hybridized on one filter to counts on the other filter. Letting R be the ratio of T7 counts to λ counts, R = (counts on T7 filter)/(counts on λ filter), we determined specificity by measuring the change in R as polymer was added to the DNA mixture. For a T7-specific polymer R should decrease relative to the ratio, R_0, for a sample without polymer, while for a λ-specific polymer R should increase relative to R_0.

Those portions of each assay sample which were precipitated with acid 10 min after the addition of cocktail were filtered through Whatman GF/C filters. The filters were washed 5 times with 3 mL of cold 1 N HCl and 0.1 M sodium pyrophosphate and finally with 3 mL of cold 95% ethanol. The filters were dried, added to vials with Aquasol, and counted in a scintillation counter.

These counts were a measure of total transcription, and the percent inhibition achieved at any polymer concentration was calculated by

$$\% I = \left(1 - \frac{\text{total transcription with polymer}}{\text{total transcription without polymer}}\right) \times 100$$

Results and Discussion

Dyes Are Selectively Incorporated into Polymers. In agreement with the findings of H. Bünemann, N. Dattagupta, and W. Müller (unpublished experiments), we found that dyes are incorporated into polymers in a higher proportion than they represent in the reaction mixture, compared to the nonspecific "spacers" such as MA. The properties of eight selected polymers are listed in Table I along with the relative proportion of each of the three monomers in the polymer. Polymers are enriched in AMG and APNR over MA by about a factor of

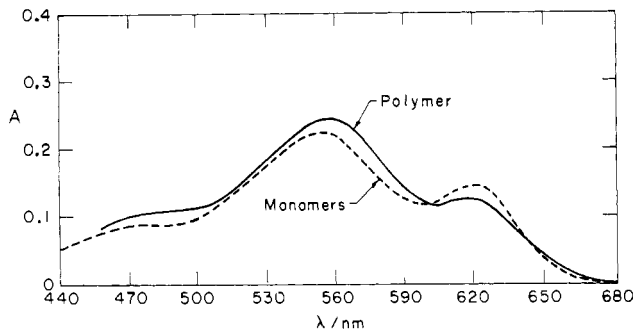


FIGURE 2: Representative visible absorption spectra of dye mixture before (monomers) and after (polymer) polymerization. Absorbance (A) of solutions containing 2.5% sodium dodecyl sulfate was measured on a Cary 14 spectrophotometer. The values are plotted vs. wavelength (λ) in nanometers. The monomer mixture contained 1.32 μM AMG, 4.19 μM APNR, and 2.9 mM MA. The estimated concentrations of monomers in the polymer sample were 1.25 μM AMG, 4.30 μM APNR, and 0.56 mM MA.

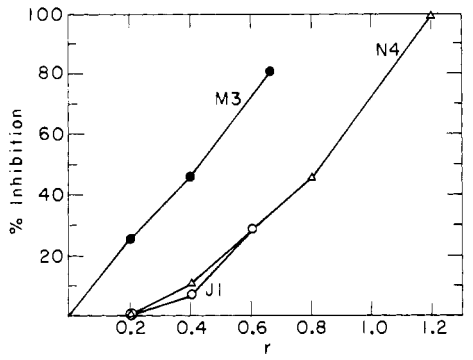


FIGURE 3: Percent inhibition of total transcription vs. concentration of polymeric dyes. Concentration of polymeric dyes is given as r, where $r = 1$ is equivalent to an acrylamide concentration 1.5 times the concentration of DNA base pairs, or ~0.4 μM dye concentration. Percent inhibition was measured as described under Materials and Methods. The data include results obtained for three polymer preparations: N4, J1, and M3, reassociated with a DNA mixture either by equilibrium dialysis (N4 and J1) or by stepwise dilution (M3).

2–10 relative to the composition of the reaction mixture. Figure 2 shows a representative visible absorption spectrum of the polymer mixture (in 2.5% $NaDodSO_4$) before and after polymerization. The two spectra are not significantly different, indicating that polymerization produces no gross change in the absorbance properties of the component dyes once aggregation or stacking effects are overcome by the addition of detergent.

The monomer ratios given for the polymers in Table I reflect the average composition summed over the population and may not accurately represent the composition of those polymer molecules with highest binding affinity or specificity. Since the polymers were synthesized on sonicated phage DNA templates ~1600 base pairs long, it is apparent that polymer preparations contain many individual molecules, each "complementary" to different DNA sequences.

Polymers Inhibit RNA Synthesis When the Dye Concentration Is in the Micromolar Range. Figure 3 shows the percentage inhibition of total RNA transcription produced by increasing the concentration of polymeric dyes. We have chosen to represent the polymer/DNA ratio in terms of the estimated fractional coverage of DNA by the polymer. Model-building studies indicate that three acrylamide residues can span ~2 base pairs. Therefore, when r, the relative number of ligands to DNA base pairs, is equal to 1, the total $-CH_2CHCONR-$ concentration is 1.5 times the total DNA

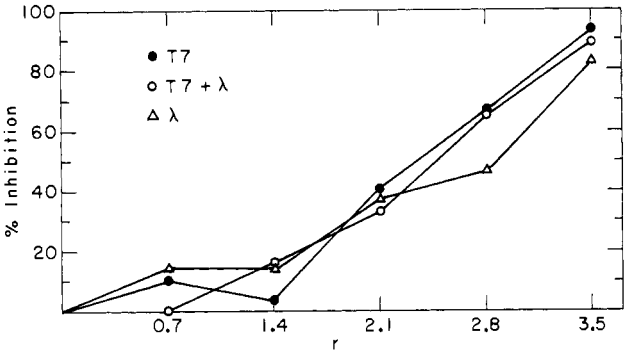


FIGURE 4: Percent inhibition of total transcription vs. concentration of dye–polymer. The same polymer preparation, D3, was assayed simultaneously on T7 DNA, λ DNA, and a mixture of the two. Although D3 was prepared on a λ DNA template, it is equally capable of inhibiting transcription of T7 DNA when assayed on either template alone.

base pair concentration. Since the total DNA concentration in the assay is 12 μg/mL (~18 μM in DNA base pairs) and the average polymer contains ~1 dye/70 acrylamide residues, the total dye concentration when $r = 1$ is 1.5 × 18 μM/70 or ~0.4 μM. Figure 3 shows that mRNA synthesis is substantially inhibited at r values less than 1, indicating that micromolar concentrations of polymeric dye residues produce strong inhibition of transcription. Monomeric dye concentrations orders of magnitude larger are required before comparable inhibition can be detected.

Gradient Dialysis Produces Variable Inhibition, Probably Due to Loss of Polymer. Figure 3 illustrates a persistent problem that plagued our experiments. The degree of binding, r, is calculated from the input ratio of polymer/DNA at the beginning of the salt gradient dialysis (samples N4 and J1). A control experiment was done in which the salt concentration was reduced by stepwise dilution in a test tube (sample M3). Only the latter showed a linear relationship between r and the percentage inhibition which passed through the origin with 0% inhibition at $r = 0$. Samples N4 and J1 and virtually all other samples tested exhibited a lag in the inhibition curve, with only slight inhibition for low values of r. We ascribe this phenomenon to variable loss of polymer during gradient dialysis. The polymer concentration was too low to verify this interpretation directly by spectrophotometric measurements. Isotopically labeled monomeric dyes were not readily available to us, so we decided to carry out these exploratory experiments without the complication of a radioactive synthesis. However, it is evident that future work must utilize dyes which can be detected at submicromolar concentration.

Polymers Inhibit mRNA Synthesis Directed by Both Bacteriophage DNA Samples. We found that the ability of a polymer to inhibit mRNA synthesis is not limited to the template on which the polymer was synthesized. Figure 4 shows the results of an experiment in which polymer sample D3 (prepared on λ DNA) was combined separately with T7 DNA, λ DNA, and a 1:1 mixture of the two. Nearly the same percentage inhibition was observed in all cases. Polymers do not appear to possess any template preference when tested on a single DNA template, and the total level of inhibition was also the same for the mixture of the two templates. Since the polymers are such tightly binding DNA ligands (N. Dattagupta, unpublished experiments), it is reasonable that all added polymer is bound. Hence, the total inhibition of mRNA production should be proportional to the fractional coverage of the DNA and should be independent of the DNA source. The phenomenon of relatively strong nonspecific DNA binding

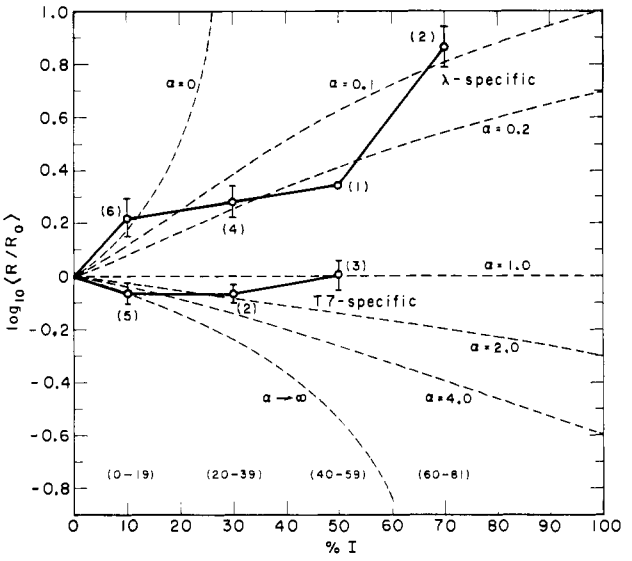


FIGURE 5: Percent inhibition (% I) dependence of average values of the ratio R (transcription from T7 DNA to transcription from λ DNA) divided by R_0, the value of R found when no polymer is added. All data from Table II are shown as log $\langle R/R_0\rangle$, where R/R_0 was averaged for percent inhibition (% I) values falling within the ranges 0–19%, 20–39%, etc. λ-Specific polymers were synthesized on λ DNA, and T7-specific polymers were synthesized on T7 DNA. The dashed lines are theoretical curves for a model in which transcription is proportional to the fraction of DNA not covered by polymer. The quantity α is the ratio of binding constant for T7 DNA divided by the constant for λ DNA, ranging from 0 (totally λ specific) to ∞ (totally T7 specific). The numbers in parentheses adjacent to each point represent the number of data points averaged to calculate the value graphed. The error bars correspond to the estimated standard deviation of the mean of each set of averaged values.

is also exhibited by naturally occurring repressors.

Some Polymers Can Selectively Inhibit mRNA Production Directed by Their Own Synthesis Template. In order to test the ability of polymers to recognize the template on which they were synthesized, we developed the competition assay described under Materials and Methods. Briefly, the polymer was annealed by salt gradient dialysis with a 1:1 mixture of λ and T7 DNAs. Relative inhibition of template function was determined from the ratio of labeled mRNA counts annealed to single-stranded T7 or λ DNA bound to nitrocellulose filters. The results of this assay for eight separate polymers are reported in Table II and summarized in Figure 5. As seen from Table II, the percentage inhibition of total mRNA synthesis at a given r varies considerably. However, there is a strong tendency for polymers prepared on λ DNA to be specific for inhibition of λ mRNA production. This is demonstrated by the R/R_0 values, where R is the ratio of T7 mRNA to λ mRNA produced in the presence of dye polymer and R_0 is the control ratio in absence of polymer. Polymers prepared on λ DNA preferentially inhibit λ mRNA synthesis, causing R to increase as r increases. Hence, in this case R/R_0 is generally greater than 1. Correspondingly, most of the R/R_0 values are less than 1 for samples prepared on T7 DNA as template. Thus, these polymers clearly are selective in their inhibitory action.

All data from Table II are assembled in averaged form in Figure 5, where log $\langle R/R_0\rangle$ values are plotted as a function of the percentage inhibition of mRNA synthesis. (Percent inhibition was chosen as the independent variable because it probably reflects most accurately the extent of polymer coverage of the DNA.) In Figure 5, the samples are grouped within ranges of percentage inhibition and the error bars represent the estimated standard deviation of the mean, calculated from the observed standard deviation of R/R_0 divided by the square root of the number of samples in each inhibition interval. It is clear that template specificity appears more pronounced for λ DNA samples than for T7 DNA samples. This may be due to a generally greater sensitivity of λ promoters to inhibition. The main qualitative point of Figure 5 is the distinct difference in average R/R_0 values for polymer samples synthesized on λ or T7 DNA templates.

Table II: Inhibition Specificity of Dye Polymers

polymer	DNA prepared on	r	% I	R	R/R_0
N4	T7	0.0	0	1.91	1.0
		0.4	3	1.55	0.81
		0.8	46	2.56	1.34
		0.0	0	3.33	1.0
		0.2	8	2.43	0.73
		0.4	46	2.73	0.82
F3	T7	0.0	0	3.12	1.0
		0.2	47	2.74	0.88
J1	T7	0.0	0	3.35	1.0
		0.4	7	3.12	0.93
		0.0	0	3.02	1.0
		0.2	20	2.10	0.69
		0.4	17	2.54	0.84
		0.6	26	3.11	1.03
Ag2	T7	0.0	0	3.20	1.0
		4.0	12	3.88	1.21
		20.0	11	2.39	0.75
Ag1	λ	0.0	0	3.20	1.0
		6.0	20	4.38	1.37
		10.0	28	8.95	2.80
M3	λ	0.0	0	0.46	1.0
		0.1	13	1.35	2.93
		0.8	69	3.99	8.67
		0.0	0	0.50	1.0
		0.66	81	3.07	6.14
D2	λ	0.0	0	2.03	1.0
		1.0	7	2.42	1.19
		2.0	12	4.70	2.31
D3	λ	0.0	0	2.03	1.0
		2.0	5	3.16	1.0
		3.0	37	3.82	1.88
		4.0	57	4.45	2.19
		0.0	0	3.33	1.0
		1.4	0	3.96	1.19
		2.1	14	2.39	0.72
		2.8	37	4.78	1.44

The dashed lines in Figure 5 show the results expected from a simple binding specificity model for inhibition, described below. A primary variable in the model is the ratio (α) of the binding constant of polymer to T7 DNA divided by the binding constant to λ DNA. The λ-specific polymers appear to bind roughly 5–10-fold better (α between 0.2 and 0.1) to λ DNA than to T7 DNA, whereas the T7-specific polymers are very roughly twice ($\alpha = 2$) as strongly bound to T7 DNA as to λ DNA. Notice that for both classes of polymers the data at low percentage inhibition are consistent with very high specificity ($\alpha = 0$ and ∞). Hence, for low degrees of binding the polymers may be much more specific than is reflected in the average estimated values of $\alpha \simeq 0.15$ and $\alpha \simeq 2$. However, our present assay method is not able to determine the specificity accurately when the percentage inhibition is low.

Reassay of the polymers listed in Table II showed that the inhibition specificity is reproducible. In other words, once a specific polymer is obtained it is reproducibly specific. However, not all of the polymers which we synthesized were specific, and again this fact could be reproducibly assayed. Usually, the failure to achieve a specific polymer could be traced to one of the components of the polymerization mixture, especially the quality of the DNA template. Hence, we

conclude that rebinding specificity *can* be achieved by template synthesis of dye polymers. However, further experiments are necessary before we can fully understand all the variables which influence the nature of the product of the template synthesis reaction.

Approximate Quantitative Model. We describe here a simple model used to estimate from the transcription data the relative binding affinity of polymers for T7 and λ DNA. The basic assumption of the model is that the rate of transcription is proportional to the fraction of each DNA molecule not covered by polymer; polymer-bound sections are assumed not to participate in transcription. Let X_1 be the fraction of T7 DNA free of polymer and X_2 the corresponding fraction for λ DNA. Then we set the ratio of transcription rates R, normalized by the value R_0 when $X_1 = X_2 = 1$, equal to X_1/X_2:

$$R/R_0 = X_1/X_2 \tag{1}$$

because the relative transcription rates are proportional to the amount of free DNA. In addition, letting

$$\alpha = K_{T7}/K_\lambda \tag{2}$$

be the ratio of binding constants of the polymer for the two DNAs, we can set

$$\alpha = \left[\frac{\text{(covered T7)}}{\text{(free T7)(free polymer)}}\right] \Bigg/ \left[\frac{\text{(covered }\lambda\text{)}}{\text{(free }\lambda\text{)(free polymer)}}\right] = \left[\frac{(1 - X_1)C_{T7}^\circ}{X_1 C_{T7}^\circ C_f}\right] \Bigg/ \left[\frac{(1 - X_2)C_\lambda^\circ}{X_2 C_\lambda^\circ C_f}\right] \tag{3}$$

where C° is the total DNA concentration and C_f is the free polymer concentration. Equation 3 simplifies to

$$\alpha = \frac{(1 - X_1)X_2}{X_1(1 - X_2)} \tag{4}$$

We can also express the fractional inhibition of RNA synthesis, $f = (\%\ I)/100$, in terms of X_1 and X_2. The ratio of synthesis rates is an average, weighted by the amount R_0 of T7 mRNA produced per unit of λ mRNA when no polymer is present. Thus

$$\frac{\text{RNA synthesis } (r)}{\text{RNA synthesis } (r = 0)} = \frac{X_2 + R_0 X_1}{1 + R_0} \tag{5}$$

Therefore, the fractional inhibition is

$$f = 1 - \frac{X_2 + R_0 X_1}{1 + R_0} \tag{6}$$

Equations 1, 4, and 6 can be combined to yield

$$\frac{R}{R_0} = \frac{1}{2\alpha R_0}(-\rho + \sqrt{\rho^2 + 4\alpha R_0}) \tag{7}$$

where

$$\rho = (\alpha - 1)(1 + R_0)f + 1 - \alpha R_0 \tag{8}$$

Equations 7 and 8 allow one to calculate R/R_0 as a function of f for any chosen α. Examples are shown in Figure 5. R_0 was set equal to 2.4, the average of the values in Table II. Only when α is very small or very large does R/R_0 depend strongly on R_0. The evident lack of mirror symmetry between the curves for $\alpha = 0$ and $\alpha \to \infty$ in Figure 5 is due to the fact that $R_0 \neq 1$. However, in the cases where α is not very large or near zero, the theoretical curve can be roughly approximated by the linear relationship

$$\log \langle R/R_0 \rangle \simeq f \log (1/\alpha)$$

which is, to a first approximation, independent of the value of R_0 at a given R/R_0 ratio.

Conclusions

Our results show that it is possible to synthesize highly active polymers which preferentially inhibit transcription directed by the DNA species used as template for production of the polymeric drug molecules. The relative binding affinity (α) can be shifted by at least an order of magnitude by changing from one DNA template to the other in the polymer synthesis. It is possible that the specificity effects are even much larger than this when the percentage inhibition of transcription is small. These results offer a new approach to improvement of the therapeutic specificity of DNA-binding drugs.

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