THE INTERCHANGEABILITY OF STIMULATORY FACTORS ISOLATED FROM THREE MICROBIAL RNA POLYMERASES[1]

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The DNA-dependent RNA polymerases of three bacteria were resolved by phosphocellulose chromatography into minimal RNA polymerases and stimulatory factors. The latter had no intrinsic RNA polymerase activity but promoted the synthesis of RNA by the minimal enzyme. Under some conditions, the factor from one species of bacteria was able to stimulate the minimal enzyme from a heterologous species.

INTRODUCTION

The DNA-dependent RNA polymerases of several bacteria have now been characterized with regard to their subunit structure (3, 8, 10). Each of these enzymes can be resolved into two major functional subunits: a high molecular weight "core" or "minimal enzyme" which catalyzes RNA synthesis and consists of several dissimilar polypeptides, and a low molecular weight monomer referred to as the "sigma factor". The latter stimulates and specifies the activity of the minimal enzyme by promoting initiation of RNA synthesis and restricting transcription to specific regions of the template DNA (1, 4, 7, 12, 13).

In the course of studies on the RNA polymerases of Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli, we have resolved a low molecular weight factor from each enzyme which stimulates the homologous minimal polymerase. In this report, we have examined the specificity of the interaction of these factors with minimal enzymes by testing the ability of the factors to stimulate RNA synthesis by heterologous minimal polymerases.

MATERIALS AND METHODS

Cultures of B. subtilis and E. coli grown in a glucose-minimal salts

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medium supplemented with 1% peptone (11) and P. aeruginosa grown in a citrate-minimal salts medium(9), were harvested by centrifugation at mid-log phase and stored at -70 ° until needed. The RNA polymerases from these bacteria were purified 100-200 fold by the Burgess method (2) or by a modification of the method of Chamberlin and Berg (5). Fractions from DEAE-cellulose or DEAE-sephadex columns were desalted by passage through Biogel A-15 and resolved by chromatography on phosphocellulose according to Burgess (2) using step elution with buffer containing 0.05 M increment increases in KCl concentration. The minimal polymerase from each of the three bacteria was eluted by buffer containing 0.45 M KCl. The stimulatory factors were noted by different KCl concentrations. Although occasional variations were noted in elution profiles, the B. subtilis factor was found in the last portion of the fractions which were not adsorbed (the flow-through), the P. aeruginosa factor was eluted by 0.15 M KCl and that of E. coli by 0.25 M KCl.

The RNA polymerase activity was assayed in 0.5 ml reaction mixtures containing: 0.05 μC 8-C^{14}-ATP, 0.4 μmole each of ATP, GTP, CTP and UTP, 25 μmoles of Tris buffer (pH 7.9), 5 μmoles of $MgCl_2$, 6 μmoles of 2-mercaptoethanol and 10-20 μg of DNA. Potassium chloride, factor preparation (2-10 μg protein) and enzyme fraction (10-30 μg protein) were added as desired; the reaction mixture was incubated at 37 C for 15 min, 100 μg bovine serum albumin was added and the reaction was terminated by addition of trichloroacetic acid. Precipitates were collected on Whatman GF/C glass filters, washed, dried and counted in a liquid scintillation counter.

DNA from P. aeruginosa phage E 79, B. subtilis phage β22 and E. coli phage T4 were extracted with cold buffered (pH 7.2) redistilled phenol, spooled by addition of ethanol, redissolved in 0.015 M NaCl - 0.0015 M sodium citrate, dialyzed against the same NaCl - sodium citrate solution and used without further purification.

RESULTS AND DISCUSSION

As shown in Table 1, the RNA polymerases of B. subtilis, E. coli and P.

Table I

RNA POLYMERASE ACTIVITY OF FRACTIONS FROM PHOSPHOCELLULOSE

Source of Polymerase	Phospho-cellulose Fraction	CPM C^{14} AMP Incorporated		
		T4 DNA	B22 DNA	E79 DNA
E. coli	(a) Minimal enzyme	308		
	(b) Factor	38		
	Combined a&b	2,627		
B. subtilis	(a) Minimal enzyme	501	79	
	(b) Factor	20	20	
	Combined a&b	1,478	1,950	
P. aerugi-nosa	(a) Minimal enzyme	220		180
	(b) Factor	40		40
	Combined a&b	1,550		1,690

aeruginosa were dissociated by phosphocellulose chromatography into two fractions: one fraction ("minimal enzyme") had low activity on native phage DNA templates, while the second fraction ("factor") had no intrinsic polymerase activity but when combined with the minimal enzyme stimulated the latter several fold. It should be noted that similar results were obtained with the B. subtilis and P. aeruginosa polymerases whether T4 DNA was used as the template or whether a correlated DNA was used such as that of B. subtilis phage β22 or P. aeruginosa phage E79. The data presented in Table I (and also in Tables II-IV) were obtained by addition of 2-10 μg of factor protein; significantly higher stimulations were observed with larger amounts of factor.

The interchange of the factor preparations was complicated by differences in the salt requirements of the three RNA polymerases. As shown by So et al. (13) and Fuchs et al. (6), the RNA polymerase of E. coli is greatly stimulated by monovalent cations, particularly by potassium. This effect is shown in Figure 1 which also demonstrates that the P. aeruginosa enzyme was similarly stimulated by KCl. As noted earlier (11) and as shown in Figure 1, the B. subtilis enzyme was inhibited by monovalent cations. This inhibition was observed at all stages of purification.

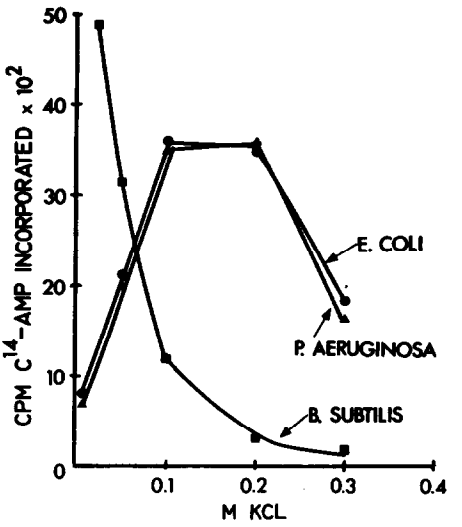

Fig. 1 The effect of KCl on the synthesis of RNA by three RNA polymerase.

Each reaction mixture contained 30 γ of protein from a DEAE-cellulose column fraction.

Table II

Effect of Adding Heterologous Factors to *E. coli* Minimal Polymerase

Factor Added	M KCl	CPM C^{14} AMP Incorporated
None	0.05	407
E. coli	0.05	635
B. subtilis	0.05	1,461
P. aeruginosa	0.05	817
None	0.2	785
E. coli	0.2	3,780
B. subtilis	0.2	8,634
P. aeruginosa	0.2	4,019

In view of these KCl effects, the three factors were tested with homologous and heterologous minimal enzymes at low and high concentrations of KCl. Experiments without KCl were not tried, since the phosphocellulose fractions contained this salt. Table II demonstrates that the E. coli minimal enzyme was only slightly stimulated by the addition of heterologous and homologous factors at low concentrations of KCl, whereas significant stimulation was observed by all three factor preparations in the presence of 0.2 M KCl. These latter results indicate that the factors from the three bacteria were interchangeable when tested with E. coli minimal enzyme.

Qualitatively similar results were obtained with the P. aeruginosa minimal enzyme as shown in Table III. As with the E. coli enzyme, the minimal polymerase was stimulated by heterologous and homologous factor preparations and this stimulation was maximized in the presence of 0.2 M KCl.

The B. subtilis minimal polymerases (Table IV) revealed a different pattern. First, the stimulatory effect of the homologous factor was greatest at low

Table III

Effect of Adding Heterologous Factors to P. aeruginosa Minimal Polymerase

Factor Added	M KCl	CPM C^{14} AMP Incorporated
None	0.05	576
P. aeruginosa	0.05	1,792
E. coli	0.05	805
B. subtilis	0.05	1,392
None	0.2	537
P. aeruginosa	0.2	2,793
E. coli	0.2	1,300
B. subtilis	0.2	1,688

Table IV

Effect of Adding Heterologous Factors to
B. subtilis Minimal Polymerase

Factor Added	M KCl	CPM C^{14} AMP Incorporated
None	0.05	295
B. subtilis	0.05	2,068
E. coli	0.05	290
P. aeruginosa	0.05	469
None	0.1	237
B. subtilis	0.1	1,257
E. coli	0.1	288
P. aeruginosa	0.1	674
None	0.2	260
B. subtilis	0.2	133
E. coli	0.2	252
P. aeruginosa	0.2	565

concentrations of KCl. Secondly, only slight stimulation was obtained with one of the heterologous factors (the *P. aeruginosa* factor), and the *E. coli* factor had no effect. A highly purified *E. coli* sigma factor preparation (kindly provided by G. Hager and Dr. B. Hall) gave similar results. Experiments conducted at an intermediate cation concentration (0.1 M KCl) also failed to show stimulation by the *E. coli* factor and again yielded only slight stimulation with the *P. aeruginosa* factor. On the other hand, Losick and Sonnenshein (10) reported a stimulation of the *B. subtilis* minimal enzyme by the *E. coli* sigma factor. It is not known at the present time whether this descrepancy with respect to the effect of the *E. coli* factor is due to differences in methods of enzyme isolation, properties of the minimal enzyme and/or factors or to differences in the cation requirements of enzymes isolated from different strains. The present experiments showing that the *B. subtilis* minimal polymerase can be significantly stimulated only by the homologous factor preparation

while the B. subtilis factor can stimulate heterologous enzyme suggests that in the interaction of minimal polymerases and factors, more restrictions are imposed by the specificity of the polymerase than by the factors.

The factor preparations obtained from the three species of bacteria studied in this investigation have the following similarities: they are resolved from 100-200 fold purified polymerases by chromatography on phosphocellulose, although they are eluted by different concentrations of KCl. Sedimentation of the preparations in glycerol or sucrose gradients yielded estimated molecular weights of approximately 90,000 for the E. coli and P. aeruginosa factors and 60,000 for the B. subtilis factor. The preparations appear to be free of DNAase activity as judged by the fact that incubation of the factor preparations with radioactive DNA for 18 hours at 37 C failed to solubilize any TCA-precipitable material.

In agreement with the findings of Bautz et al. (1), the product synthesized by the minimal enzyme of E. coli in the absence of added sigma factor was symmetrical and had a low molecular weight. The three factors isolated in our experiments, when added to E. coli minimal polymerase, promoted the synthesis of similar product RNAs. These RNAs were asymmetric, had high molecular weights and similar profiles on sucrose gradients; moreover, the three in vitro RNAs gave identical results in RNA-DNA competition-hybridization experiments where the competitor RNA was T4 "early" RNA isolated 5 minutes after phage infection (details will be presented elsewhere).

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