HYBRID RNA POLYMERASES FORMED FROM CORE ENZYMES AND SIGMA FACTORS OF *E. COLI* AND THERMOPHILIC *B. MEGATERIUM*

V.G. NIKIFOROV

Kurchatov Institute of Atomic Energy, Moscow, USSR

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1. Introduction

RNA polymerase from thermophilic *Bacillus megaterium*, unlike the enzyme from mesophilic *Escherichia coli*, is practically unable to initiate RNA synthesis at 20°. The rate of RNA synthesis catalyzed by thermophilic *B. megaterium* polymerase is 20–40 times lower at 20° than at 30°. If, however, initiation is performed at high temperature the rate of subsequent RNA synthesis at 20° is only half that at 30° [1, 2]. To determine which of the two RNA polymerase components, core enzyme or sigma factor, is responsible for inability to initiate RNA synthesis at 20°, we formed hybrid RNA polymerase molecules from these two components of *E. coli* and thermophilic *B. megaterium* enzymes and measured the activities of hybrids at 30° and 20°.

2. Methods

RNA polymerase was purified from thermophilic *B. megaterium* as described previously [1] and from *E. coli* according to Chamberlin and Berg [3]. The preparations obtained were of about 50% purity. Core enzyme and sigma factor were separated by phosphocellulose chromatography according to Burgess et al. [4] with minor modifications. The RNA polymerases from *E. coli* and *B. megaterium* require different concentrations of KCl for maximal activity. Therefore in experiments with *B. megaterium* core enzyme the reaction mixture (0.5 ml) contained: tris HCl 50 μmoles pH 8.0; $MnCl_2$ 1 μmole; KCl 25 μmoles; unlabeled ATP, GTP, UTP, CTP, 0.2 μmole of each; ^{14}C-UTP; T2 DNA 50 μg. In experiments with *E. coli* core enzyme, 2.5 μmoles $MgCl_2$ were added and the amount of KCl increased to 60 μmoles. The amount of core enzyme in an experiment was 2–4 μg, sigma factor was added at nearly saturating concentrations. The samples were incubated for 20 min, fixed and washed as described in [1].

3. Results and discussion

RNA polymerase from thermophilic *B. megaterium*, like that from other bacteria, may be separated on a phosphocellulose column into two main fractions: core enzyme and sigma factor. The core enzyme is less active than the holoenzyme with T2 DNA and DNA from *B. megaterium* as templates. The activity of the core enzyme is stimulated by sigma factor (table 1). It should be

Table 1

Stimulation of core enzyme from *B. megaterium* by sigma factor from *B. megaterium* at 40°.

Enzyme preparations	^{14}C-UMP incorporation (counts/100 sec)	
	T2 DNA	DNA from *B. megaterium*
Holoenzyme	5260	3400
Core enzyme	330	180
Sigma factor	180	80
Core + sigma	5140	1480

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Table 2
Stimulation of core enzyme from *E. coli* by sigma factors from *E. coli* and *B. megaterium* at 30° and 20°.

Enzyme preparations	^{14}C-UMP incorporation (counts/100 sec)			
	Exp. 1		Exp. 2	
	30°	20°	30°	20°
(a) Core *E. coli*	1130	580	500	430
(b) Sigma *E. coli*	640	250	170	100
a + b	6650	3580	4420	2390
(c) Sigma *B. megaterium*	430	10	100	0
a + c	4630	610	3970	410
a + c + b	7500	1520	4910	1290

Table 3
Stimulation of core enzyme from *B. megaterium* by sigma factors from *B. megaterium* and *E. coli* at 30° and 20°.

Enzyme preparations	^{14}C-UMP incorporation (counts/100 sec)					
	Exp. 1		Exp. 2		Exp. 3	
	30°	20°	30°	20°	30°	20°
(a) Core *B. megaterium*	480	5	100	15	350	50
(b) Sigma *B. megaterium*	1340	30	160	2	2250	60
a + b	5270	50	1050	45	6020	230
(c) Sigma *E. coli*	230	110	120	110	480	510
a + c	2120	260	610	220	2720	600

noted that separation of these two components of *B. megaterium* RNA polymerase may be achieved only at low temperature. If chromatography is performed at room temperature about 50% of the initial RNA polymerase activity is eluted from phosphocellulose, and it is only slightly stimulated by sigma factor. Polyacrylamide gel electrophoresis of the core enzyme from *B. megaterium* dissociated by 1% SDS, performed according to Shapiro et al. [5], reveals only three main bands: two poorly resolved slow bands and one fast band in positions corresponding to *E. coli* β', β and α subunits, respectively. The same analysis of RNA polymerase eluted from phosphocellulose at room temperature reveals one additional band of intermediate mobility, probably corresponding to the sigma subunit. So RNA polymerase components from thermophilic *B. megaterium* have the same polypeptide compositions as reported for RNA polymerase from *E. coli* [4].

E. coli and *B. megaterium* are phylogenetically distant species and their RNA polymerases do not share common antigenic determinants as shown by immunodiffusion analysis. Nevertheless the core enzymes from these two species may be stimulated not only by homologous sigma factors but also by heterologous ones (tables 2 and 3). Similar data on the interchangeability of sigma factors were recently reported for *E. coli, Pseudomonas aeruginosa* and *B. subtilis* [6]. It may thus be supposed that at least the contact surfaces of core enzymes and sigma factors have a common structure in different bacteria.

As can be seen from table 2, sigma factor from *B. megaterium* stimulates the core enzyme from *E. coli* at 30°, but not at 20°, while sigma factor from *E. coli* stimulates at both temperatures. Preincubation of *E. coli* core enzyme and sigma factor from *B. megaterium* with DNA at 30° does not stimulate RNA synthesis at 20°. It may be concluded that the inability to initiate RNA synthesis at 20° depends on the sigma factor. Yet another possible explanation is that *B. megaterium* sigma factor merely fails to form a stable complex with *E. coli* core enzyme at 20°. If so, the presence of *B. megaterium* sigma factor should not have interfered with stimulation of *E. coli* core enzyme by homologous sigma factor at 20°. However, *B. megaterium* sigma factor in fact markedly inhibits stimulation of *E. coli* core by *E. coli* sigma factor at 20° (table 2). This suggests that at 20° *B. megaterium* sigma factor does attach to *E. coli* core enzyme but this complex fails to initiate RNA synthesis at low temperature like holoenzyme from *B. megaterium*. The inhibitory effect of *B. megaterium* sigma factor which is observed only at low temperature may be explained by competition with *E. coli* sigma factor for the attachment site on the core enzyme.

If the inability to initiate RNA synthesis at 20° depends only on sigma factor it should be expected

that the activity of hybrid RNA polymerase formed from *B. megaterium* core and *E. coli* sigma factor will differ at 20° and 30° only by a factor of two. However, core enzyme from *B. megaterium* with both homologous and heterologous sigma factor is far less active at 20° than at 30° (table 3). Hence it may be concluded that *B. megaterium* core enzyme was not 'taught' by *E. coli* sigma factor to initiate RNA synthesis at low temperature. It should be pointed out, however, that in six of the seven experiments performed with four independent enzyme preparations, sigma factor from *E. coli* was somewhat more active in stimulating *B. megaterium* core at 20° than *B. megaterium* factor, while at 30° homologous factor was always more effective. Therefore it may be supposed that the dominance of *B. megaterium* RNA polymerase properties in hybrid molecules is not complete and that sigma factor from *E. coli* facilitates initiation at low temperature to some extent. To verify this supposition further experiments with more purified sigma factor preparations are required, since stimulations observed at 20° are comparable to the residual activity present in sigma preparations used.

From the data presented in this report it may be concluded that inability of *B. megaterium* RNA polymerase to initiate RNA synthesis at low temperature is determined by both its components, core enzyme and sigma factor. It has been supposed that during initiation RNA polymerase undergoes conformational changes [7]. Our results suggest that initiation requires changes both in the core enzyme and sigma factor since the presence of at least one of these two components from *B. megaterium* in a hybrid molecule prevents RNA initiation.

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