A NEW FORM OF RNA POLYMERASE ISOLATED FROM ESCHERICHIA COLI

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SUMMARY

Stationary E. coli cells contain a second form of RNA polymerase separable from the standard enzyme by DEAE chromatography. The new form is almost inactive with natural DNA templates, it is active with poly dAT. It requires Mn^{++}, Mg^{++} works less well. The activity is sensitive to rifampicin. RNA synthesis by the standard RNA polymerase using T4 or E. coli, but not T7, DNA as template is inhibited by this second form of RNA polymerase. This in vitro repressor-like effect suggests an in vivo role of RNA polymerase in regulating RNA synthesis.

In B. subtilis, RNA synthesis for vegetative growth, and for sporulation is carried out each by a different form of the B. subtilis RNA polymerase[1,2]. E. coli, too, has different physiological states--log and stationary phases of growth. The standard E. coli holo RNA polymerase as described by Burgess[3] can be isolated from either log or stationary cells though the former yield a more active extract. We show here that E. coli extracts, especially from stationary phase cells, contain a second form of RNA polymerase which we will call polymerase II. The properties of this enzyme show it is different from standard E. coli RNA polymerase (polymerase I), core or holo enzyme, and suggest that it is not a new enzyme but more likely an altered form of the standard enzyme. Polymerase II is almost inactive in vitro with natural DNA templates but can interfere with RNA synthesis by polymerase I on such DNA. It may be that this in vitro repression by polymerase II reflects an in vivo regulatory role.

MATERIALS AND METHODS

Two isogenic cell lines were used. A derivative of E. coli W3110 requiring arginine and tryptophan (arg^-, $tryp^-$) was a gift of Dr. C. Yanofsky. A rifampicin resistant (arg^+, $tryp^-$) mutant was obtained by transduction[4].

Superbroth contains 32g tryptone, 20g yeast extract, 5g NaCl, 5 ml 1N NaOH, 1 l water (personal communication from Dr. H. Lodish). Whatman DE23 cellulose was used for DEAE column chromatography. Sepharose 6B was from Pharmacia. Egg white lysozyme and DNase I were from Worthington. Rifampicin (Rif.) was from Calbiochem and ^{3}H-UTP was from New England Nuclear. Calf thymus DNA (type I) and poly dAT were from Sigma Chemical Co. T4 and T7 phage DNA and E. coli DNA were prepared by phenol extraction.

RNA polymerase activity was assayed according to Burgess[3] except that 0.1 μg T4 DNA and ^{3}H-UTP (9,700 cpm/n mole) were used and the assay volume was 0.1 ml. Polymerase II activity was assayed by the same procedure except that 1 mM $MnCl_2$ and 0.1 μg poly dAT were used instead of $MgCl_2$ and T4 DNA. Potassium phosphate (0.4 mM, pH 7.5) was included in the assay mixture to inhibit polynucleotide phosphorylase which contaminates early stages of enzyme purification[3]. Assays were carried out at 37° for 10 min. and stopped by adding 1 ml cold H_2O followed by 3 ml of 5% cold CCl_3COOH. The precipitates were collected on Whatman glass fiber filters and further washed with CCl_3COOH, followed by 95% ethanol. The filters were dried and counted in a toluene based scintillator.

RESULTS AND DISCUSSION

Preparation of Cells and Enzymes. E. coli cells were grown overnight in super broth to 14 OD_{550} units and harvested by low speed centrifugation. Harvested cells are washed in Tris-EDTA buffer (0.01 M Tris·HCl, pH 7.9, 0.001 M EDTA) and pelletted.

The washed cells are suspended in 5 volumes of Tris-EDTA buffer and lysozyme (0.1 mg/ml) is added to the cold suspension. Thirty minutes later the suspension is frozen and then thawed. This mixture is adjusted to buffer A concentration (0.01 M Tris·HCl, pH 7.9, 0.001 M EDTA, 0.001 M dithiothreitol, 0.01 M $MgCl_2$, and 5% glycerol) according to Burgess[3] and treated with DNase

Abbreviation: Poly dAT, polydeoxyadenylic-thymidylic copolymer.

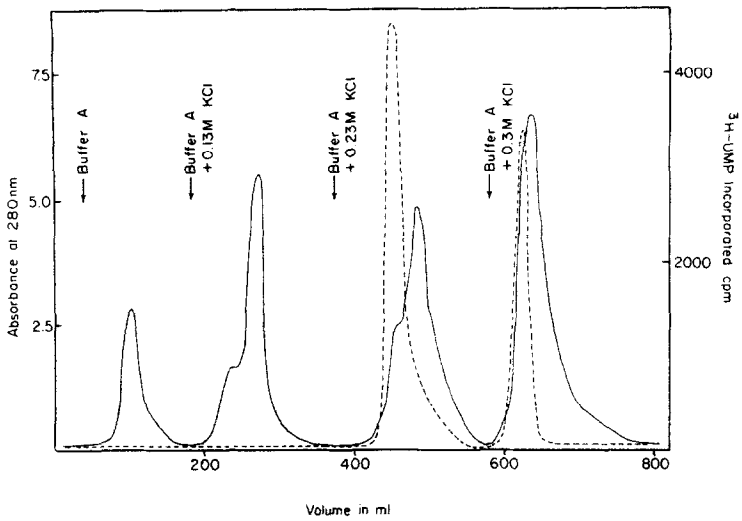

Figure 1. DEAE-cellulose column chromatography of RNA polymerase. The standard RNA polymerase eluted at 0.23 M KCl is assayed with the standard assay mixture. The activity eluted at 0.3 M KCl is assayed with poly dAT and $MnCl_2$ assay mixture.

(3 μg/ml) for 30 min. in the cold to remove DNA. Cell debris are removed by 20 min. centrifugation at 12,000 x g and ribosomes are removed by a second centrifugation of 2 hr. at 150,000 x g. The supernatant is used for enzyme purification. The proteins precipitating between 33% and 55% saturation with $(NH_4)_2SO_4$ (pH 7.0) are collected by centrifugation. This precipitate is dissolved in buffer A and dialyzed overnight against buffer. The dialyzed sample is applied to a DEAE column previously equilibrated with buffer A. Proteins are eluted with a KCl gradient in buffer A. (This gradient can be continuous or discontinuous.) The standard RNA polymerase is eluted at 0.23 M KCl as described by others[3,5], and the second peak of weak activity is eluted at 0.3 M KCl (Fig. 1). The two peaks of RNA polymerase activity are each precipitated with 55% $(NH_4)_2SO_4$. The precipitate is dissolved in minimum amount of buffer A containing + 0.2 M KCl and dialyzed against the same buffer overnight. The dialyzed sample is chromatographed on a Sepharose 6B column which has been previously equilibrated with buffer A + 0.2 M KCl. The results of Sepharose chromatography indicate that polymerase I and polymerase II are not separable

Table 1. The requirement of poly dAT directed RNA synthesis by polymerase II.

Components	^{3}H-UMP incorporated (cpm)
Complete	1480
- poly dAT	46
- ATP	44
- enzyme	41
- $MnCl_2$	135
- $MnCl_2$ + 20 mM $MgCl_2$	366
- KCl	454

The complete assay mixture contains 1.25 μg poly dAT and 3 μg polymerase II.

Table 2. Template specificity of polymerase I and polymerase II.

Templates	^{3}H-UMP incorporated (cpm)	
	Polymerase I	Polymerase II
Poly dAT (1.25 μg)	3180	1332
Calf thymus DNA (2 μg)	1630	116
T4 DNA (1 μg)	2552	203
T7 DNA (1 μg)	820	47
E. coli DNA (1 μg)	626	38

Polymerase I (3 μg) is assayed with the standard assay mixture. Polymerase II (3 μg) is assayed with the $MnCl_2$ assay mixture.

on this column but other proteins are removed. The activity peak is collected and purified by a second cycle of DEAE column chromatography and used in the subsequent experiments.

Properties of Polymerase II. Unlike RNA polymerase holoenzyme or core enzyme, polymerase II requires Mn^{++} for activity instead of Mg^{++} (Table 1 and Fig. 2). The poly dAT directed RNA synthesis by polymerase II is dependent on template, ATP and KCl (Table 1). There is no lag period, thus incorporation is not unprimed synthesis[6]. The salt optimum for polymerase II reaction is 0.2 M KCl.

The size of polymerase I and II are similar—they are inseparable by gel

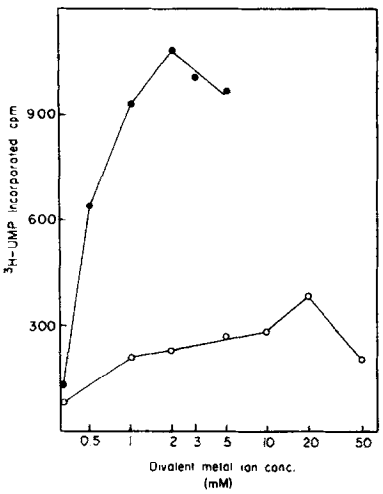

Fig. 2.

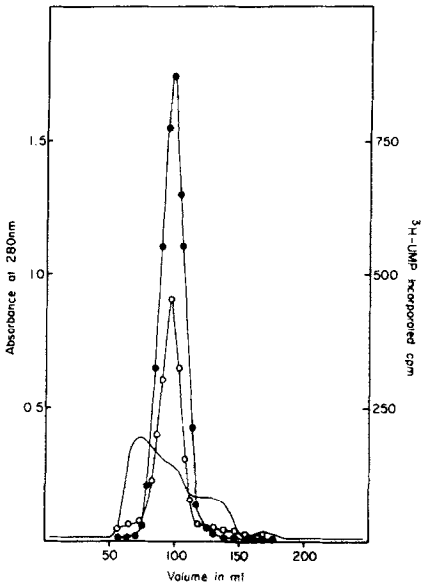

Fig. 3.

Figure 2. Stimulation of polymerase II activity by divalent metal ions. Activity is assayed as in Table 1. •——•, $MnCl_2$ stimulated incorporation; o——o, $MgCl_2$ stimulated incorporation.

Figure 3. Co-chromatography of polymerase I and polymerase II on Sepharose 6B column. Activity is assayed as in Fig. 1. •——•, polymerase I activity; o——o polymerase II activity.

filtration: Rif sensitive polymerase I and rif resistant polymerase II were mixed and cochromatographed on Sepharose 6B. Polymerase I was assayed with T4 DNA on which polymerase II is inactive. Polymerase II was assayed in the presence of rif to inhibit polymerase I and Mn^{++} was used as cofactor. Figure 3 shows that both activities coincide. This similarity in size, together with the fact that polymerase II like polymerase I is sensitive or resistant to rif depending on the allelic form of the arginine H linked gene of rif sensitivity (β subunit) suggests that polymerase II is likely an altered form of polymerase I. However, more work is needed to prove this.

Polymerase II as a Repressor. Table 2 shows that polymerase II is active with poly dAT but not with natural DNA. It is not a good transcriptase, but it might be a repressor. This idea is based on several observations: Rif sensi-

Table 3. The inhibition of polymerase I activity by polymerase II.

Templates	Polymerase II (μg)	Polymerase I (μg)	^{3}H-UMP incorporated (cpm)
T4 DNA (0.1 μg)	0	12	1567
	3	12	1547
	6	12	1386
	9	12	1170
	12	12	839
T7 DNA (0.25 μg)	0	12	584
	3	12	668
	6	12	706
	9	12	716
	12	12	672
E. coli DNA (0.25 μg)	0	12	568
	3	12	456
	6	12	428
	9	12	388
	12	12	312

Template DNA are first preincubated at 37° for 3 min. with various amounts of polymerase II as indicated. 12 μg of polymerase I and $MgCl_2$ assay mixture is then added to each tube and further incubated for 10 min. before CCl_3COOH precipitation.

tivity in E. coli is dominant over resistance in merodiploids and in vitro. Ilyina, et al.[7] have shown that RNA synthesis by a mixture of rif sensitive and resistant polymerase I is rif sensitive. They call the rif sensitive polymerase I a rif dependent repressor which blocks the DNA promoters and thus initiation by the rif resistant polymerase I. Recently, it was proven that rif inactivated polymerase I retains the ability to bind to DNA[8]. Dunne, Bautz and Bautz[9] showed that the T3 phage induced RNA polymerase bind to but does not transcribe T7 DNA. They suggest that T3 excludes coinfecting T7 phage by this mechanism.

We preincubated polymerase II with a limited amount of DNA and then added polymerase I and the assay mix. The results (Table 3) show RNA synthesis is inhibited with T4 and E. coli—but not T7—DNA. Presumably, polymerase II binds to the former but not the latter DNA. The simplest, but novel, explana-

tion is that polymerase acts like a repressor. Such repression is slightly different from the sense of Jacob and Monod[10] in that it acts directly on the promoters. It would be a general repressor of RNA synthesis depending on the affinity for various promoters. We suppose that polymerase I and II may be interconvertible, a change that is sensitive to the cells metabolism and that can regulate the rate of transcription.

Two additional observations may be relevant. (1) We find that the ratio of polymerase II to polymerase I increases as cells approach stationary phase. (2) T7 but not T4 phage grow on stationary E. coli cells: A dense lawn was formed on Nutrient Agar plates in nine hours at 37°C after seeding with 2 x 10^8 log phase cells. Four drops of either T4 or T7 containing 10, 10^2, 10^3 or 10^5 phage were put on the lawn which was then incubated overnight. None of the T4 and all of the T7 spots developed. These observations fit but do not prove the proposed role of polymerase II. Further work is in progress.

ACKNOWLEDGMENTS

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