DNA-DEPENDENT RNA POLYMERASE FROM PHAGE T4 INFECTED E. COLI: AN ENZYME MISSING A FACTOR REQUIRED FOR TRANSCRIPTION OF T4 DNA

by

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SUMMARY

The "modified" DNA-dependent RNA polymerase, isolated from E. coli cells infected with phage T4 and unable to transcribe T4 DNA, can be activated by addition of a protein factor normally associated with RNA polymerase from uninfected cells.

It was found recently that the DNA-dependent RNA polymerase from E. coli can be further separated into a basic polymerase and a protein factor by chromatography on a phosphocellulose column (Burgess et al., 1968). The basic polymerase is active with calf thymus DNA as template, but fails to transcribe phage T4 DNA appreciably. Addition of the protein factor to the basic polymerase fully restores the template activity of T4 DNA.

Walter et al. (1968) have reported the isolation of a modified E. coli RNA polymerase from cells infected with phage T4. This enzyme shows a drastically reduced activity with T4 DNA as template,

but is quite active with DNA from calf thymus. In this respect, the "modified" (T4) polymerase is strikingly similar to our basic E. coli polymerase. It was therefore of interest to see whether addition of the protein factor obtained from the E. coli RNA polymerase would render the T4 enzyme capable of transcribing T4 DNA.

In this communication we show that the T4 polymerase can be made to transcribe T4 DNA by addition of E. coli factor, suggesting that the modification due to infection results in a loss of factor from the RNA polymerase either in vivo or upon breakage of the cells. We also show by acrylamide gel electrophoresis that the T4 polymerase is indeed lacking the factor normally associated with the E. coli enzyme.

MATERIALS AND METHODS

E. coli RNA polymerase was purified from E. coli K12 (λ) cells according to the method of Burgess (cited in Burgess et al., 1968) up to the DEAE step. Further purification was accomplished by chromatography on a column of T4 DNA bound to cellulose (Alberts et al., 1968) the polymerase was adsorbed in 0.15 M KCl and eluted with 0.6 M KCl. Free factor and basic polymerase were obtained by chromatography on phosphocellulose as described (Burgess et al., 1968).

T4 RNA polymerase: 600 liters of E. coli B, grown at 37° C to 8×10^8 cells/ml, were infected with T4am82 at a multiplicity of 5. After 40 minutes, harvest of the cells was begun by centrifugation through a Sharples centrifuge and the culture was chilled another 20 minutes later to 10° C. Harvest was completed after 3 hours. The T4 RNA polymerase was purified from 400 g of infected cells as the E. coli polymerase, except that a 1.5 fold concentration of DNAase was used to remove the more resistant T4 DNA from the crude extract.

Assays for RNA polymerase were done according to Chamberlin and Berg (1962), each tube containing in a total volume of 0.5 ml (in μ moles): Tris, pH 7.9 (20); 2-mercaptoethanol (5); KCl (75); $MgCl_2$ (0.6); 120 μ moles each of the four nucleoside triphosphates, with ^{3}H-ATP as the labeled substrate (spec. act. 800 cpm/μmole); 30 μg of DNA; and enough enzyme to incorporate 2 to 10 μmoles of AMP into TCA insoluble polynucleotides during 10 minutes of incubation at 37° C.

The following abbreviations were used: DC enzyme = RNA polymerase obtained by elution with 0.6 M KCl from the DNA-cellulose column, PC enzyme = basic RNA polymerase eluted from a phosphocellulose column with 0.35 M KCl at pH 7.9; F = protein factor obtained from the flow-through of the phosphocellulose column run and required for enzymatic activity of PC enzyme with T4 DNA as template.

Table 1

Activity of different polymerase preparations with T4 and calf thymus DNA in the presence and absence of factor (F)

mμ moles of ^{3}H AMP incorporated in 10 min.

Source of enzyme		μg protein per assay	Template DNA T4 -F	Template DNA T4 +F (6 μg)	Template DNA calf thymus -F	Template DNA calf thymus +F (6 μg)
E. coli	DC	30	6.3	8.0	9.6	12.0
E. coli	PC	30	0.9	10.6	5.0	13.0
T4	DC	50	0.2	5.8	2.8	5.8
T4	PC	30	0.1	5.4	2.8	6.8
none			< 0.1	0.5	< 0.1	0.7

RESULTS AND DISCUSSION

Table 1 compares the activities of E. coli and T4 polymerase with T4 and calf thymus DNA as template in the presence and absence of factor. It is clear that both the T4 DC and the T4 PC enzyme resemble E. coli PC rather than E. coli DC enzyme in their dependence on factor to transcribe T4 DNA. Thus it is tempting to conclude that the T4 enzyme is in fact basic polymerase which for some reason has lost F on breaking of the cells. That factor is lost already in crude extracts is apparent from the data of Walter et al. (1968) which show that T4 DNA is transcribed poorly even in crude extracts. Similarly, we have observed that addition of F to crude extracts of infected cells restores template activity to T4 DNA (data not shown here).

The time dependent RNA synthesis catalyzed by PC enzyme from uninfected and from infected cells in the presence and absence of F with T4 DNA as template is shown in Figure 1. Whereas traces of factor still appear to be associated with the E. coli PC enzyme, T4 PC appears to be completely devoid of factor. From the ratio of the slopes we can estimate that F stimulates the E. coli PC enzyme 21 fold, whereas the activity of the T4 PC enzyme is increased more than 70 fold by the addition of F. So far our best T4 PC preparation could not be stimulated by F to exactly the same specific activity as E. coli PC (Table 1, Figure 1), although specific activities of up to 60% of E. coli PC have been observed. Whether this lower activity is a consequence of an altered α subunit (Figure 2) remains to be investigated.

The E. coli factor has been identified by acrylamide gel electrophoresis in 8 M urea or in 0.1% sodiumdodecylsulfate (SDS) as a single component (band σ) moving between bands β and α, the major

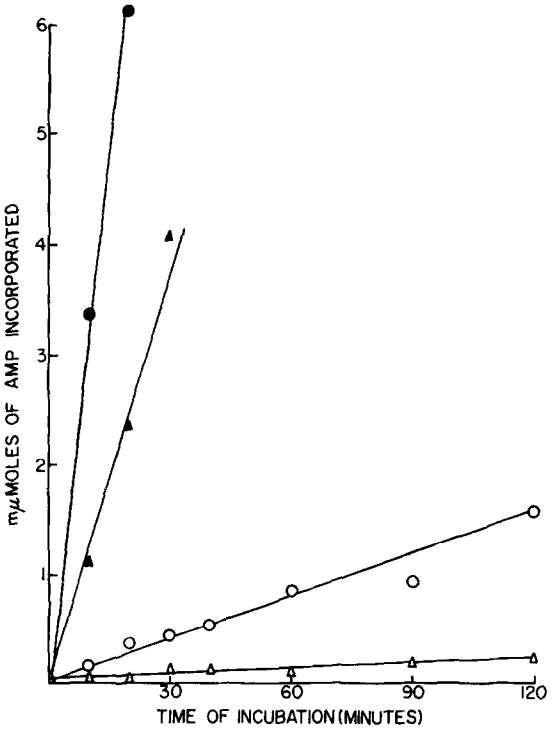

Figure 1. Incorporation of ^{3}HAMP into acid insoluble polynucleotides as a function of incubation time with 7.5 μg of either E. coli PC or T4 PC enzyme plus or minus 3 μg of factor.

○ : E. coli PC - F; ● : E. coli PC + F; △ : T4 PC - F;

▲ : T4 PC + F.

components of basic polymerase (Burgess et al., 1968). As shown in Figure 2, band σ is present in E. coli DC enzyme, but absent in E. coli PC, T4 DC, and T4 PC enzyme. From the intensity of the individual bands we estimate that E. coli DC is about 80 - 90, T4 DC about 70 per cent pure and E. coli PC and T4 PC are both better than 90 per cent pure polymerase. The position of the α bands of E. coli and T4 polymerase differ slightly, indicating that the lack of band σ may not be the only difference between E. coli and T4 polymerase. When the two DC or the two PC enzymes are

mixed one obtains two α bands, the more slowly moving band apparently belonging to the T4 protein, the faster moving band representing the

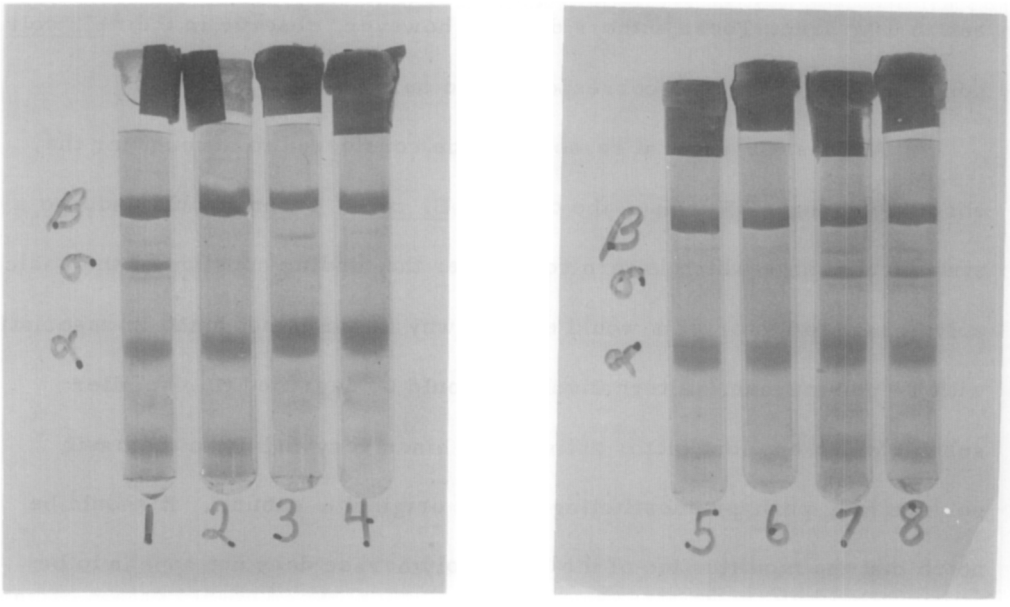

FIGURE 2

Polyacrylamide gel electrophoresis analysis of preparations of RNA polymerase from infected and non-infected E. coli.

gel #1 25 μg DC (E. coli)
gel #2 25 μg PC (E. coli)
gel #3 25 μg DC (T4)
gel #4 25 μg PC (T4)
gel #5 12.5 μg PC (E. coli) + 12.5 μg PC (T4)
gel #6 12.5 μg PC (E. coli) + 6 μg PC (T4)
gel #7 12.5 μg DC (E. coli) + 12.5 μg DC (T4)
gel #8 12.5 μg DC (E. coli) + 6 μg DC (T4)

Gels contain 0.1% SDS and 5% acrylamide and were run at a current of 6.5 m Amp. per gel for 3.5 hours (Shapiro et al., 1967).

Staining and destaining of the gels was according to Burgess et al., (1968).

α band of E. coli. Thus, it appears that, as a consequence of infection, component α becomes modified to (or replaced by) a polypeptide of slightly higher molecular weight (in SDS gels, polypeptides move faster with decreasing molecular weight). The change in position of the α band is probably the same as that observed originally by Walter et al. (1968) in gels run in 6 M urea. These authors did not, however, observe in their E. coli polymerase a component corresponding to our σ band.

At present, several reasons can be considered to account for the observed changes. For one, the original E. coli α subunit could undergo a structural change which in turn could alter the binding constant of the basic polymerase to factor; this would explain why factor is not found in association with T4 polymerase. Alternatively, F could be degraded to a smaller subunit which has lost factor activity but binds very tightly to the basic polymerase, perhaps substituting for the original α subunit. It should be noted that the modification of the basic polymerase does not appear to be complete, since especially the T4 DC enzyme seems to show both α bands (Figure 2). Loss of factor, however, appears to be complete under our conditions, since no σ band can be detected in the T4 DC enzyme.

The RNA produced on a T4 DNA template by both E. coli and T4 enzymes in the presence of factor hybridizes well with T4 DNA and is largely asymmetric. The question whether T4 PC + F transcribes different T4 genes than the combination of E. coli PC + F is currently being investigated.

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