# MODIFIED DNA-DEPENDENT RNA POLYMERASE FROM E.COLI INFECTED WITH BACTERIOPHAGE $T_4$

G. Walter, W. Seifert, W. Zillig

Max-Planck-Institut für Biochemie
München 15, Goethestr.31, Germany

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It has been shown by Buchanan and Sköld (1964) that the DNA-dependent RNA polymerase activity in crude extracts from E.coli measured using DNA from $T_4$-phage as template shows a strong decrease within a few minutes after $T_4$-phage infection. Protein synthesis is required for this inactivation. The authors, and later Furth and Pizer (1966), ascribed this phenomenon to the appearance of an inhibitor. It has been demonstrated by Hall (1967) that this inactivation appears much less pronounced when calf thymus DNA is used as the template. In this case activity even increases again at later times. It will be shown in this communication that these effects may be attributed to a structural modification of E.coli polymerase as a consequence of phage infection (G.Walter, W.Seifert, W. Zillig, 1967).

## Materials and Methods

Phage $T_4$ am 82 was multiplied on E.coli CR 63. Cultures of E.coli B ($5 \cdot 10^8$ cells/ml in full medium) were infected at a multiplicity of 5, poured onto ice 20 minutes after infection and harvested. RNA polymerase was isolated using the procedure described previously (Zillig et al., 1966; Fuchs et al., 1967). The last electrophoretic purification step was, however, replaced by a second centrifugation using a sucrose gradient

containing an additional 0.5 M $NH_4Cl$ so that the enzyme sediments with 13S.

The RNA polymerase assay has been described (Zillig et al., 1966; Fuchs et al., 1967). DNA's from phage $T_4$ and $T_3$ and from E.coli were prepared by the phenol method. Non glucosylated $T_4$-phages were grown on E.coli K12 strain W4597, which was a generous gift from Dr. S. E. Luria. Calf thymus DNA was purchased from Sigma Chemical Company. A potent antiserum against purified RNA polymerase from E.coli was prepared using conventional methods and freed of nucleases by precipitation with ammonium sulfate (0-45 % saturation).

## Results and Discussion

As shown in Fig. 1 the polymerase activity in crude extracts from infected cells measured using $T_4$-DNA as template has reached its minimum level 5-10 minutes after infection. With calf thymus DNA as the template activity decreases in the first five minutes then increases again to a plateau value. The differential curve shows the appearence of an altered activity.

Polymerase was prepared from cells harvested 20 minutes after infection. At this time the alteration was complete. The activity of this purified enzyme, "$T_4$-polymerase" measured in DNA saturation has been compared with that of the polymerase from non-infected cells using different DNA's as templates (Fig. 2). With $T_4$-DNA (either glucosylated or non-glucosylated) $T_4$-polymerase activity is a twentieth of that of E.coli polymerase, but with calf thymus or E.coli DNA more than half and with DNA from $T_3$-phage a third (summary in Table 1).

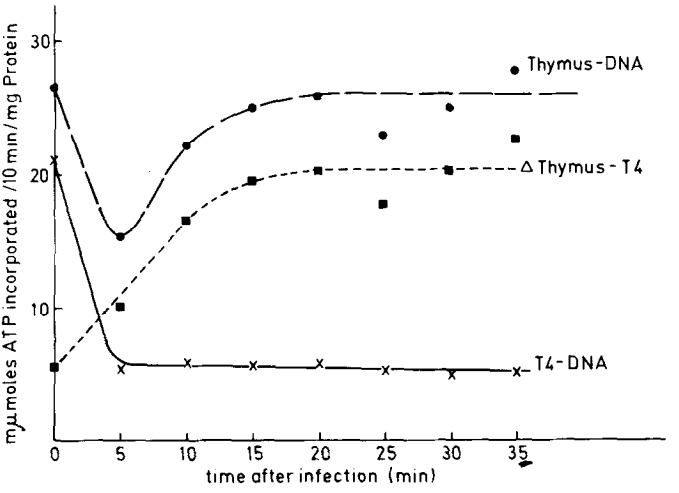


Fig. 1: Activity of RNA polymerase in crude extracts of $T_4$-infected cells at different times after infection using $T_4$-(——x——) and calf thymus DNA (— — —). E.coli B was grown to $6 \cdot 10^8$ cells/ml and infected with $T_4$ am 82 at an m.o.i. of 5. Samples were taken at different times after infection; extracts were prepared by lysis with lysozyme and tested for polymerase activity.

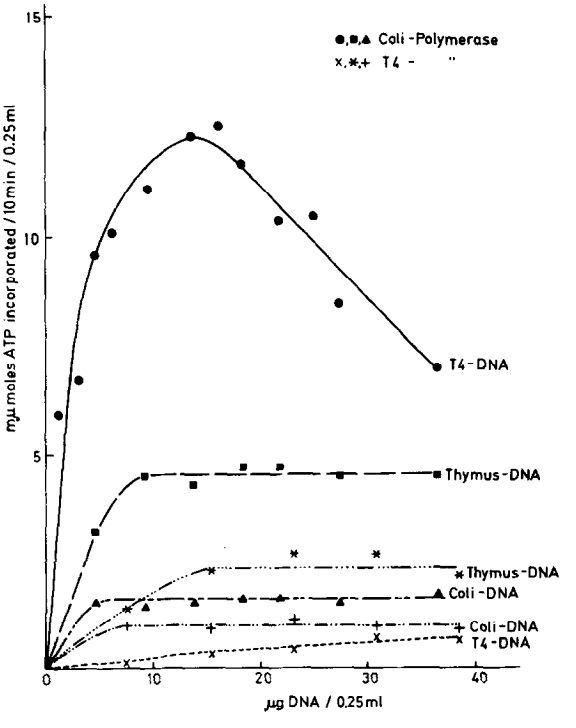


Fig. 2: Activity of equal amounts of E.coli-polymerase and "$T_4$-polymerase"(5μg each) using increasing amounts of $T_4$-, calf thymus and E.coli-DNA.

Table 1: Ratios of activities of E.coli- and $T_4$-polymerase with $T_4$-, $T_3$-, calf thymus- and E.coli-DNA's as templates. 5µg of enzymes and saturating amounts of DNA's were used.

| | E.coli-Enzyme mµ moles ATP incorporated | $T_4$-Enzyme mµ moles ATP incorporated | Enzyme ratio E.coli-/$T_4$-polymerase |
|---|---|---|---|
| $T_4$-DNA | 12,2 | 0,6 | 20,3 |
| calf thymus DNA | 4,6 | 2,6 | 1,8 |
| E.coli-DNA | 1,5 | 1,0 | 1,5 |
| $T_3$-DNA | 12,0 | 4,0 | 3,0 |
| DNA ratio $T_4$/calf th. | 2,7 | 0,23 | |
| DNA ratio $T_4$/E.coli | 8,1 | 0,6 | |
| DNA ratio $T_4/T_3$ | 1,0 | 0,15 | |

For E.coli polymerase $T_4$-DNA is a twofold more efficient template than calf thymus DNA, but for "$T_4$-polymerase" $T_4$-DNA is a fivefold less efficient template than thymus DNA. The ratio of template efficiencies for the "$T_4$-enzyme" does not change throughout purification. Therefore, no inhibitor has been removed and the alteration appears to be a property of the pure enzyme.

The "$T_4$-polymerase" elutes from the DEAE column at the same position as the E.coli enzyme, is precipitated by ammonium sulfate at the same saturation, sediments with about 24S at low ionic strength and with about 13S at high ionic strength (0.5 M $NH_4Cl$) and, therefore, is very similar to the

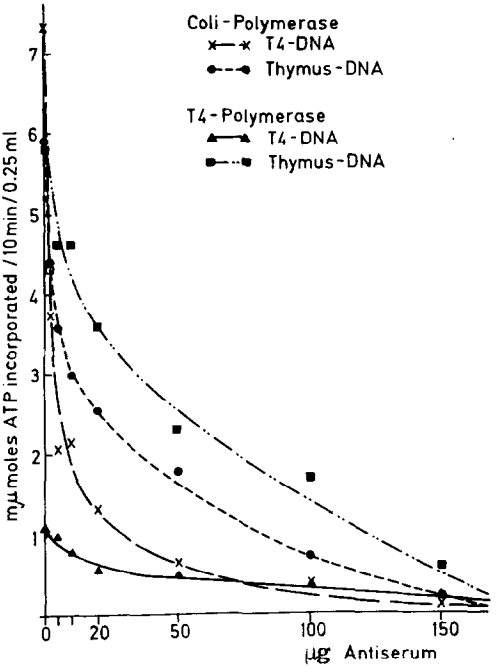


Fig. 3: Inactivation of E.coli- and "$T_4$-polymerase" (5 μg each) by antibodies against E.coli polymerase, measured using saturating amounts of calf thymus- and $T_4$-DNA's as templates. Enzymes were preincubated with the amounts of antiserum shown on the abscissa for 30 min. at 37°C; RNA-synthesis was then initiated by the addition of DNA, triphosphates and magnesium ions.

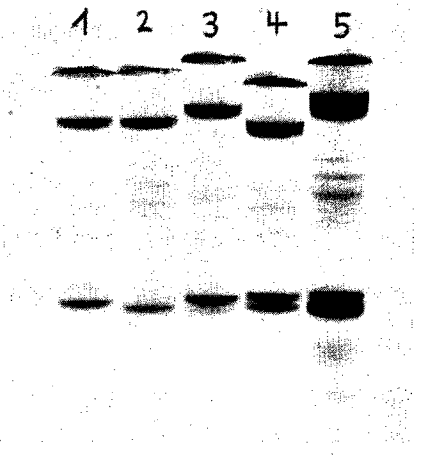


Fig. 4: Polyacrylamide disc electrophoresis in 6 M urea at pH 9.5 with
1) 7,5 μg E.coli B-polymerase
2) 7,5 μg "$T_4$-polymerase" (RNA-polymerase from $T_4$-inf.E.coli B)
3) 7,5 μg E.coli B-polymerase + 3,25 μg "$T_4$-polymerase"
4) 7,5 μg E.coli B-polymerase + 7,5 μg "$T_4$-polymerase"
5) 7,5 μg E.coli B-polymerase + 30 μg "$T_4$-polymerase"

E.coli enzyme in these features.

As shown in Fig. 3, equal amounts of purified antiserum against E.coli polymerase are required for total inactivation of equal amounts of E.coli- and $T_4$-enzyme of comparable purity. The difference in the initial activities of both enzymes with $T_4$- and calf thymus DNA follows from their DNA saturation curves (Fig. 2).

No difference between the two enzymes could be demonstrated with the immunodiffusion technique of Ouchterlony nor by immunoelectrophoresis (1 % agarose, sodium barbital buffer pH 8.6, ionic strength 0.025). Thus, with three immunochemical methods "$T_4$-polymerase" has not been distinguished from E.coli polymerase. This means that at least a large part of the structure of the "$T_4$-enzyme" is either identical or very similar to that of the E.coli enzyme. RNA polymerase from Anacystis nidulans (v.d.Helm and Zillig, 1967) does not react with antiserum against E.coli polymerase. This finding supports the idea that the "$T_4$-enzyme" is a modified E.coli polymerase.

When E.coli polymerase is treated with 6 M urea at above neutral pH in the presence of mercaptoethanol it dissociates into small subunits which may be separated at pH 9.5 by disc electrophoresis in polyacrylamide gel into a fast and a slow moving main component (A and B)(E.Fuchs, 1967; Zillig, W., E.Fuchs et al, 1967). The purified "$T_4$-enzyme" also shows two main bands. In mixtures of both polymerases the faster band from the "$T_4$-enzyme" ($A_{T4}$) moves significantly ahead of the A band from E.coli enzyme (Fig. 4). Thus a structural difference in one of the subunits of the two enzymes has been demonstrated.

This difference could be the result of either 1.) a comp-

lete substitution of the bacterial A subunit by a phage gene dependent $A_{T4}$ subunit. 2.) a modification of A yielding $A_{T4}$ by either removal or addition of structural elements or by an intramolecular reaction like S-S-bonding or 3.) the non-covalent binding of a phage dependent material. This possibility appears unlikely since the complex would have to be extremely stable, against high ionic strength, 6 M urea at pH 9.5 and under the conditions of enzyme purification.

Experiments are in progress which will decide between these possibilities and which should elucidate the mechanism, by which this modification occurs.

The physiological significance of this modification remains to be clarified. In vitro using DNA from $T_4$-phage as template, it leads to the inhibition of the transscription of early genes which are the only ones transscribed in vitro by E.coli polymerase (Khesin et al., 1962, 1963; Geiduschek et al., 1966). The nature of the product synthesized by this enzyme and the possibility that newly synthesized $T_4$-DNA acts as a template are currently under investigation.

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