STUDIES OF COMPLEXES OF RNA POLYMERASE AND λ DNA*

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Several laboratories have recently reported results on the interaction of RNA polymerase with template molecules. Fox, Gumport, and Weiss (1965) showed that complexes of enzyme and DNA (or RNA) could be detected by sucrose density gradient centrifugation. The complexes were formed in the absence of ribonucleoside triphosphates and divalent cation. Studies of Crawford et al. (1965) demonstrated that only a limited amount of enzyme was bound to polyoma DNA at 0° in the presence of Mg^{++}, and they suggested that the enzyme bound to DNA sites specific for the initiation of RNA synthesis. Formation of tRNA-enzyme complexes has been reported by Bremer and Stent (in press). That DNA or tRNA bound in such complexes with the enzyme is at least partially exchangeable with subsequently added DNA or RNA has been reported (Bremer and Stent, in press; Anthony, Zeszotek, and Goldthwait, 1966).

The investigation reported here concerns the formation and properties of complexes of E. coli RNA polymerase and λ DNA. Most of the studies were carried out with the use of ^{3}H-labeled enzyme. The results suggest that divalent cation, although not required for the formation of complexes of enzyme and DNA, may alter the extent of complex formation. Even under conditions of extensive RNA formation, the amount of ^{3}H-enzyme bound to DNA is the same as that bound after mixing ^{3}H-enzyme, DNA, and Mg^{++} at 0°. ^{3}H-Enzyme bound at 0° is at least partially displaced from the complexes by subsequent addition of unlabeled enzyme.

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Experimental--Unlabeled and ^{3}H-labeled RNA polymerase were prepared as described in the accompanying paper (Stevens, Emery, and Sternberger). The most purified enzyme fractions were used in all the experiments. No alteration of the sedimentation properties of λ DNA occurred upon incubation of it with the enzyme under the conditions of the experiments reported below. Possible degradation was tested for by isolating the λ DNA by phenol extraction after incubation with the enzyme and then examining its sedimentation properties before and after alkali denaturation. RNA polymerase activity was measured as described by Stevens and Henry (1964). Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

λ DNA was prepared from E. coli W 3101 (λ) according to the procedure of Kaiser and Hogness (1960).

Results and Discussion--Binding of E. coli RNA polymerase to DNA was detected using ^{3}H-labeled DNA. Sucrose density gradient centrifugation of mixtures of the DNA and enzyme showed a fast-sedimenting, labeled peak which could be shown to have RNA polymerase activity when incubated with ribonucleoside triphosphates. A second peak of free enzyme could be demonstrated at the top of the gradient tube. To determine the extent of binding, different amounts of enzyme were then mixed with 2.5 μg of λ DNA and the amount of enzyme bound determined by measuring the capacity of the fast-sedimenting DNA peak to stimulate RNA formation when incubated with the four ribonucleoside triphosphates. The first studies were carried out at 0° in the presence of Mn^{++} and the results are shown in Fig. 1. (Mn^{++} was thought to be the divalent cation of choice since it stimulates RNA formation with λ DNA 1.2-1.5-fold better than when Mg^{++} was used.) It was found that as the level of enzyme was increased, more was bound. No saturation level was reached even at the highest concentration of enzyme tested. However when Mg^{++} was substituted for Mn^{++}, less binding occurred as shown in Fig. 1. A saturation value of about 2.5 μg of enzyme per μg of DNA was

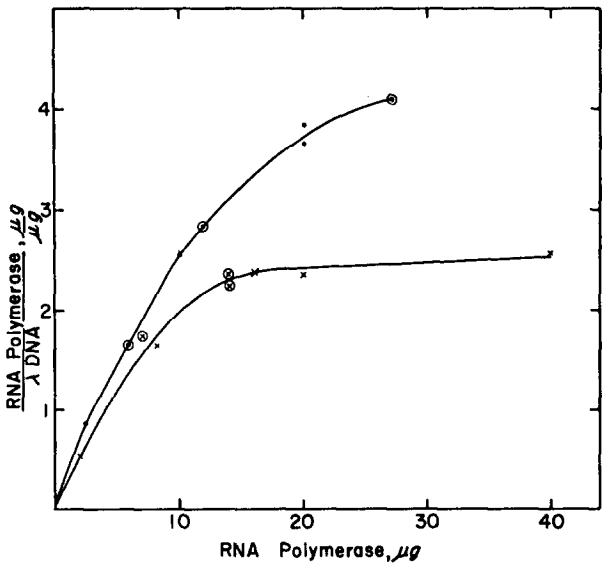

Figure 1. Extent of binding of E. coli RNA polymerase to λ DNA in the presence of Mn^{++} or Mg^{++}. The reaction mixtures contained Tris buffer, pH 7.8, 20 mM; KCl, 50 mM; $MgCl_2$ or $MnCl_2$, 2.5 mM; 2-mercaptoethanol, 5 mM; 2.5 μg of λ DNA; and enzyme, specific activity = 350, as shown, in a final volume of 0.2 ml. After 10 minutes at 0°, the mixtures were layered on a 4.6 ml linear gradient of 5-20% sucrose containing Tris buffer, KCl, Mg^{++} or Mn^{++}, and mercaptoethanol as above, and centrifuged for 60-120 minutes at 35,000 rpm (0°). Fractions were collected from the bottoms of the tubes and analyzed for RNA polymerase activity as described by Stevens and Henry (1964). Calf thymus DNA (Worthington Biochemicals Corp.) was added to each reaction tube. In the experiments in which ^3H-enzyme was used, each fraction was precipitated with 5% trichloroacetic acid. The acid-insoluble precipitates were collected on Millipore filters and counted in a toluene solvent in a Packard Tri-Carb scintillation counter. ●—●—●, Mn^{++}; x—x—x, Mg^{++}; ⊙, ^3H-enzyme with Mn^{++}; ⊗, ^3H-enzyme with Mg^{++}.

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reached. Binding could also be demonstrated in the absence of any divalent cation and the amount bound was greater than that in the presence of Mg^{++}. When 20 and 40 μg of enzyme were incubated with 2.5 μg of DNA, 2.6 and 3.5 μg, respectively, were bound per μg of DNA. Similar results on the extent of binding with Mn^{++} or Mg^{++} were obtained when ^3H-labeled enzyme was used. The amount bound was determined by measurement of rapidly-sedimenting ^3H-radioactivity. Fig. 1 shows the results (symbols enclosed in a circle) obtained with several levels of ^3H-enzyme. The results show that although the binding of the enzyme to λ DNA is not dependent on divalent

cation, the extent of binding is dependent on the divalent cation used.

With ^{3}H-labeled enzyme, the extent of binding was measured using other reaction conditions. The results are shown in Table I. In Expts. 1-3, the effect of ribonucleoside triphosphates on the binding was measured in the presence of Mg^{++} at 0°. Essentially the same amount of enzyme was bound in the presence of either three or all four ribonucleoside triphosphates. Expts. 4-6 show that the amount of enzyme bound was also the same when the mixtures were incubated at 37° in the absence or presence of ribonucleoside triphosphates. Expts. 7 and 8 compare the amount of enzyme bound at 0° in the presence of Mn^{++} (in the absence of ribonucleoside triphosphates) with that bound at 37° in the presence of Mn^{++} and all four ribonucleoside triphosphates. A greater amount of bound enzyme was detected at 0°. The value of 1.6 μg of enzyme bound per μg DNA (Expt. 8) is of the same order of magnitude as that found under identical conditions with Mg^{++}.

Further studies to determine the maximal amount of enzyme bound to DNA during incubation periods at 37° in the presence of the ribonucleoside triphosphates, i.e., when RNA synthesis takes place, are in progress. Using reaction mixtures similar to those shown in Table I (Expts. 6 and 8) the amounts of RNA formed have been determined by measuring ^{14}C-ATP incorporation.

Table I. Effect of Different Reaction Conditions on the Amount of ^{3}H-RNA Polymerase Bound to λ DNA

Expt. No.	Reaction Conditions	^{3}H-Enzyme Bound
		μg/μg DNA
1	0°, Mg^{++}	2.0
2	0°, Mg^{++}, ATP, UTP, CTP	2.2
3	0°, Mg^{++}, ATP, UTP, CTP, GTP	2.0
4	37°, Mg^{++}	2.2
5	37°, Mg^{++}, ATP, UTP, CTP	2.3
6	37°, Mg^{++}, ATP, UTP, CTP, GTP	2.4
7	0°, Mn^{++}	4.1
8	37°, Mn^{++}, ATP, UTP, CTP, GTP	1.6

In Expts. 1-6, the reaction mixtures contained 13 μg of ^{3}H-enzyme, 115 cpm/μg, and 2.1 μg of unlabeled λ DNA. In Expts. 7 and 8, the mixtures contained 26 μg of ^{3}H-enzyme, 150 cpm/μg, and 2.6 μg of DNA. ATP, CTP, UTP, and GTP, when added, were each at a concentration of 0.25 mM. All other reaction conditions were as described in Fig. 1. Both the centrifugations and the determinations of ^{3}H-enzyme were carried out as described in Fig. 1.

Extensive RNA formation occurs, the amount formed being equal to the amount of DNA added. These results suggest that the DNA template is saturated by a small amount of enzyme (2 μg/μg of DNA) either in the absence or presence of RNA synthesis. It is only in the presence of Mn^{++} at 0° that a larger amount of enzyme can be bound to the DNA.

In other experiments, the stability of the enzyme-DNA complexes to increased ionic strength has been measured. The complexes formed at 0° (Mg^{++} or Mn^{++}) were quite sensitive to ionic strength, being dissociated almost 50% by 0.10 M KCl and 100% by 0.20 M KCl. The complexes formed at 37° in the presence of all four ribonucleoside triphosphates were stable at concentrations of KCl up to 0.40 M.

That the complexes of enzyme and DNA can be isolated by sucrose density gradient centrifugation would suggest that a poorly dissociable complex is formed. As mentioned above, however, exchange of free template with enzyme-bound template has been reported. We have measured the exchange of free enzyme with labeled enzyme bound in the complexes formed at 0°. Results are shown in Table II. Labeled enzyme can be at least partially displaced

Table II. Exchange of Free Enzyme (Unlabeled) with DNA-Bound ^{3}H-Enzyme at 0°

Amount of Unlabeled Enzyme Added	^{3}H-Enzyme Bound
	μg/μg DNA
(1) None	1.8
5-Fold excess	0.7
(2) None	2.4
10-Fold excess	0.6

In (1) above, the reaction mixtures contained 13 μg of ^{3}H-enzyme and 3.1 μg of λ DNA. In (2), they contained 24 μg of ^{3}H-enzyme and 2.6 μg of DNA. Other reaction components and incubation conditions were as described in Fig. 1. After 10 minutes at 0°, unlabeled enzyme was added and the mixtures were incubated for an additional 5 minutes before centrifugation. The centrifugation conditions and determination of bound ^{3}H-enzyme were as described in Fig. 1.

from the complexes formed at 0°. Preliminary experiments show that the enzyme bound in complexes formed at 37° can also be displaced by unlabeled enzyme. These experiments will be reported elsewhere.

Exchange of both free template and free enzyme with enzyme-DNA complexes at 0° suggests that the binding reaction is reversible. However, reactions involving a dimeric enzyme (Richardson, 1966; Stevens, Emery, and Sternberger, accompanying paper) such as those illustrated below could account for the exchange reactions. Reaction (2) shows the formation of a complex of dimeric enzyme (E_m-E_m) and DNA, and reactions (3) and (4) show the exchange of free monomeric enzyme (E_m^*) and free DNA (DNA°) with the respective component in the complex. A reaction such as (3) might also account for the possible migration of the enzyme on a DNA template. This

(1) $2\ E_m \rightleftarrows E_m\text{-}E_m$

(2) $E_m\text{-}E_m + DNA \rightarrow (DNA\text{-}E_m\text{-}E_m)$

(3) $(DNA\text{-}E_m\text{-}E_m) + E_m^* \rightleftarrows (DNA\text{-}E_m\text{-}E_m^*) + E_m$

(4) $(DNA\text{-}E_m\text{-}E_m) + DNA° \rightleftarrows (DNA°\text{-}E_m\text{-}E_m) + DNA$

concept and other aspects of enzyme-DNA complexes are under further investigation.

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