REVERSIBLE ATTACHMENT OF RNA POLYMERASE TO DNA AS A FUNCTION OF TEMPERATURE

J.M. DUBERT and L. HIRSCHBEIN

(Service de Biochimie Cellulaire, Institut Pasteur, Paris)

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Available observations are in agreement with the idea that RNA polymerase attaches to native DNA at sites that are characterized by weak pairing (Berg et al., 1965 ; Jones et al., 1966 ; Maitra et al., 1966 ; Stent, 1966). If this is the case, we should expect this attachment to be significantly temperature-dependent, since secondary or tertiary structures are more stable at low temperatures (Felsenfeld et al., 1967 ; Inman, 1966 ; Follett et al., 1967). The present work brings further arguments in favor of such temperature dependence in agreement with the observations made by Walter et al., 1967).

The experimental schemes for determining conditions for attachment employs preincubation at different temperatures in the absence of nucleoside triphosphates. Under these conditions no initiation should occur. On the other hand, when three of the four nucleosides triphosphates are present during preincubation both attachment and initiation must be considered. Although the evidence is indirect, these kinetic experiments do provide information on the initial steps leading up to the transcription.

MATERIALS and METHODS

The DNA-dependent RNA polymerase was prepared from E. coli A19 (RNAse-less) by the procedure of Babinet (1967). Calf-thymus DNA was prepared according to the procedure of Kay et al. (1952), followed by digestion by pronase, treatment with lauryl sulfate and deproteinization as described by Sevag et al. (1938). Bacteriophage λ-DNA was prepared according to

the procedure of Kaiser and Hogness (1960). Denatured calf-thymus DNA was prepared by heating DNA (200 μg/ml) in buffer (Tris 10^{-2}M, pH 7.7) to 100° for five minutes, followed by rapid chilling. The poly I (Miles Chemical Co) was dialyzed for 24 hours against buffer (Tris 10^{-2}M, pH 7.5 ; NaCl 0.2 M), then against the same buffer without NaCl.

The nucleoside triphosphates were purchased from Pabst Laboratories, and the radioactive nucleoside triphosphates (^{3}H or ^{14}C) from Schwarz (Bio-Research). The specific activity of these radioactive substrates was adjusted to values varying between 1,200 and 2,500 cpm/mμmole.

Reaction mixtures (0.1 ml) contained the following : potassium-maleate buffer, 5×10^{-2}M, pH 7.5 ; β-mercaptoethanol, 4×10^{-3}M ; $MnCl_2$, 4×10^{-3}M ; nucleoside triphosphates, 4×10^{-4}M for each, one of them being radioactive and 2 to 3 μg of native or denatured DNA ; enzyme, one to two units. Incubations were carried out at 37°. Variations of these conditions are described in the legends to figures.

RESULTS and DISCUSSION

Conditions for attachment. Figure 1 shows the logarithm of the initial rate of RNA synthesis as a function of the reciprocal of the absolute temperature between 0° and 37°C. With native λ-bacteriophage DNA as template (curve A), the slope increases as the temperature decreases. Thus, the temperature coefficient (Q_{10}) for the intervals between 37° and 20°, 20° and 10°, and, 10° and 0° are 2.9, 5.8, and 10, respectively. Similar results were obtained when calf-thymus DNA, E. coli DNA or poly dAT were used as templates.

The decrease in rate of RNA synthesis is less pronounced if the template is denatured calf-thymus DNA (Figure 1, curve B) or for the synthesis of polyriboadenylate (not shown here). Heat denatured calf-thymus DNA was used instead of heat denatured λ-DNA because it does not renature under our experimental conditions in contrast to λ-DNA. The increase in the slope (absolute value) observed at low temperatures may be due either to the slowing down of the polymerization reaction or to a reduced ability to accomplish

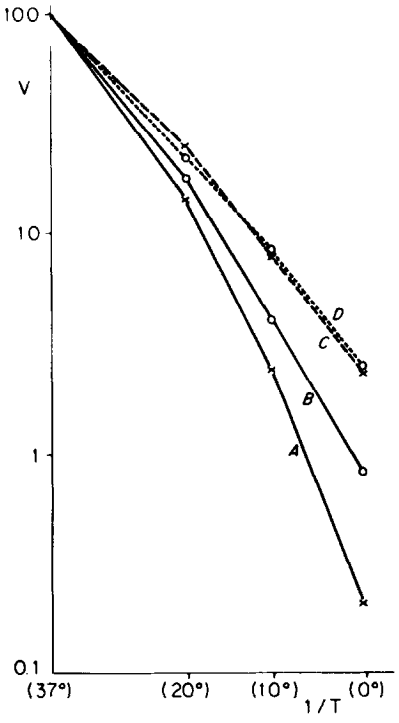

Figure 1. - Variation of initial rates of transcription as a function of temperature : effect of preincubation at 37°. Incubation under the usual conditions of the complete system with λ-DNA (curve A) or with denatured calf-thymus DNA (curve B). Curves C and D correspond to cases where preincubation of the enzyme with λ-DNA (curve C) or denatured calf-thymus DNA (curve D) were carried out for ten minutes in the absence of nucleoside triphosphates. The relative initial rates of uptake of radioactivity expressed as the amount of radioactive nucleoside monophosphate incorporated in 5 minutes, are plotted on a log scale against the reciprocal of absolute temperature. At low temperatures, the values corresponding to five minutes incubations were deduced from the kinetic curves observed for longer periods.

the initial steps of the transcription. If the attachment of the enzyme to the DNA is the limiting step for RNA synthesis at low temperatures, it should be possible to increase the rate of the reaction at low temperatures by prior attachment of enzyme to template at higher temperatures. This is confirmed by the results shown in figure 1 (curve C and D). The slope of the curves approaches a constant value for the 0°-37° interval.

The decrease in the extent of binding of the enzyme to DNA at low temperatures indicated by these experiments was confirmed by kinetic studies carried out at 0°, with and without preincubation at 37°. Such kinetic studies

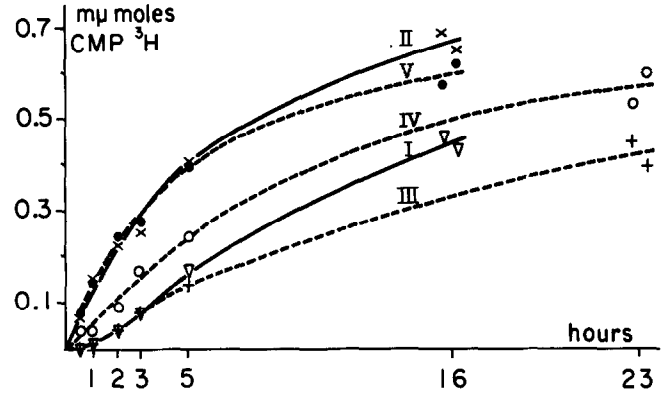

Figure 2. - Kinetics of transcription at 0° with and without preincubation of λ-DNA with RNA polymerase at 37° or at 0°. (I) Incubation at 0° without preincubation. (II) Preincubation in the absence of nucleoside triphosphates at 37° for 10 minutes or (III) at 0° for 17 hours. Preincubation in the presence of ATP, GTP and UTP ($3x10^{-4}$M) at 0° for 17 hours (IV) or at 37° for 10 minutes (V). ^{3}H-CTP with a specific activity of 1,300 cpm/mμM was added after the preincubation, and uptake of ^{3}H-CTP is presented as a function of time of subsequent incubation at 0°. Each of the four nucleoside triphosphates utilised was chromatographically free of the other three.

are reported in figure 2. The marked increase in the initial rate of transcription provoked by preincubation at 37° (curve I and II) must be attributed to the attachment which occurs during this preincubation between RNA polymerase and DNA. In the absence of nucleoside triphosphates, the binding is the only step of the transcription process which may take place. Three nucleoside triphosphates added during preincubation at 0° favor the initial steps of transcription (attachment and possibly initiation) but not if they are added during preincubation at 37° (curves IV and V).

The differential attachment of RNA polymerase to its template as a function of temperature can also be shown by utilizing the property of poly I of forming an inactive complex with enzyme not yet attached to DNA (Hirschbein et al., 1967). DNA and enzyme are preincubated for varying times at 37° or at 0°. Then poly I* is added as well as the four nucleoside triphosphates and

* The quantity of poly I used is that which results in complete inhibition of transcription when added to the complete reaction medium before the enzyme

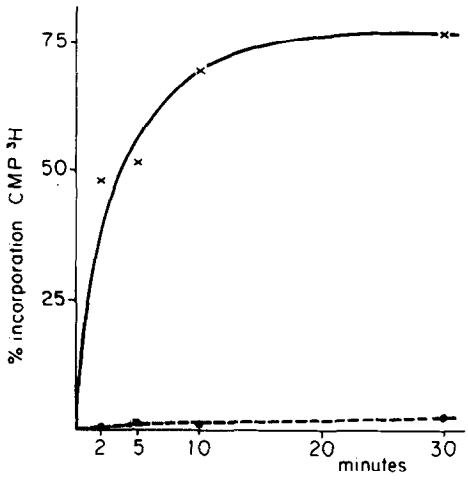

Figure 3. - Inhibition of the transcription reaction when poly I is added after preincubation of native DNA and enzyme at 37° and at 0°. Preincubation at 37° (--x--) or at 0° (--o--) of λ-DNA and enzyme in the absence of nucleoside triphosphates, then addition of poly I. After two minutes at 37°, incubation of the complete system for ten minutes at 37°. Final concentration of poly I is 8.2×10^{-5}M. The 100% incorporation is that measured in the absence of poly I and without preincubation.

incubation is carried out at 37°. When preincubation is carried out at 37° the value of the initial rate of reaction increases rapidly with time of preincubation and attains its limiting value after ten mintues (Fig.3). The effect of poly I is the same whether it is added at 0° or at 37°. The same kind of experiment was performed with denatured calf-thymus DNA instead of native λ-DNA. Preincubation in this case permits the attachment of a large portion of the enzyme, even at 0°.

Our kinetic experiments thus provide indirect evidence in favor of the hypothesis that segments of native DNA molecules whose secondary structure have a relatively weak stability constitute the preferred sites of attachment for RNA polymerase. At 37° these DNA segments have a non-paired or a weakly paired structure and the RNA polymerase can thus easily attach to them, contributing to their destabilisation. At 0° these segments have a much more stable secondary structure and the enzyme is not able to attach to them at least in the absence of nucleoside triphosphates.

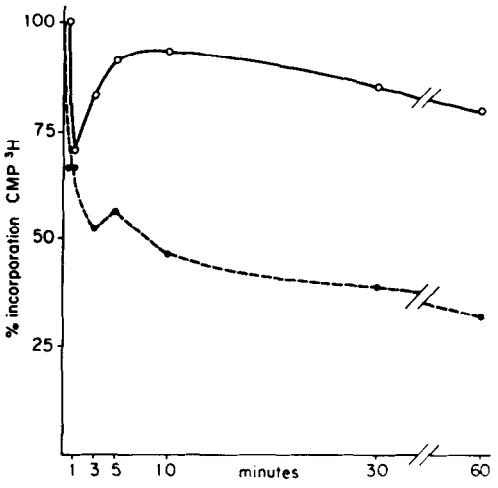

Figure 4. - Reversibility of attachment of RNA polymerase to λ DNA as a function of temperature. Enzyme and λ DNA are preincubated at 37° for 10 minutes, then the system is chilled and again preincubated at 0° for varying lengths of time (abcissa) in the presence (—o— upper curve) or in the absence (--•-- lower curve) of ATP, GTP and UTP. The system, completed with either H^3-CTP or the four nucleoside triphosphates including the radioactive CTP, is then incubated sixty minutes at 0°. Relative uptake of H^3CMP is plotted as a function of time of preincubation at 0°. If the system is not preincubated at 0° the incorporation is maximum and corresponds to 0.21 mμmoles of ^{3}H-CMP.

Reversibility of attachment as a function of temperature. The attachment of RNA polymerase to native DNA resulting from preincubation at 37° may be shown to be partly reversible when the system is brought to 0°. The decrease in activity of the system is very rapid during the first few minutes, which can be interpreted as the result of detachment of the enzyme. This detachment is not total since, even after two hours at 0°, the level of activity is greate than the one observed in the absence of preincubation at 37° (Fig.4).

Since the conditions of attachment and detachment defined in our experiments exclude appreciable transcription, both must have occured at the same site, presumably the same regions which under normal conditions have a maximum affinity for RNA polymerase.

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