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COMPARATIVE STUDY OF CALF THYMUS AND WHEAT GERM RNA POLYMERASE II : STABILITY OF INITIATION COMPLEXES AND ELONGATION RATES

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Abstract.

Pure wheat germ RNA polymerase II but not calf thymus RNA polymerase II forms relatively stable binary complexes (half life time of 30 minutes at 0°C) with superhelical SV 40 DNA. On the contrary, the addition of a specific dinucleotide and a single ribotriphosphate permits the formation of highly stable complexes between both enzymes and SV 40 DNA. The elongation of RNA chains with preinitiated wheat germ enzyme only is stimulated by sarkosyl. These observations suggest that the wheat germ enzyme, as compared to that isolated from calf thymus, may contain a protein factor, a more native structure or both that permit efficient initiation and elongation of RNA chains on double stranded DNA.

Introduction.

Studies on the mechanism of RNA synthesis with procaryotic RNA polymerases have allowed the definition of several steps involved in this process : formation of binary complexes, initiation, elongation and termination[1]. Stable enzyme-DNA complexes capable of initiating RNA synthesis in the presence of ribotriphosphates are formed at specific sites identified *in vivo* or *in vitro* as promoters[2]. The fact that the formation of stable initiation complexes requires the opening of the double stranded DNA[3] explains the increased affinity of *E.coli* RNA polymerase for superhelical DNA[4,5]. In an attempt to study RNA synthesis in eucaryotic cells, we chose to analyse the interactions between RNA polymerase II (B) and viral double stranded DNA. That the simian virus 40 (SV 40) genome is transcribed *in vivo* by RNA polymerase II is suggested by the sensitivity of viral RNA synthesis to α-amanitin[6]. This template is a useful tool for the

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study of RNA polymerase-DNA interactions because it is small and we know its complete DNA sequence[7,8] and the structure of the viral mRNA[9-11].

In the present study, we set out to better characterize the capacity of calf thymus and wheat germ RNA polymerase II to form stable initiation complexes and to elongate RNA chains with SV 40 DNA as template. We show that, contrary to the calf thymus enzyme, a fraction of the wheat germ enzyme is capable of forming a relatively stable binary complex (half life time - τ- of 30 minutes) with superhelical DNA. Initiation complexes with both enzymes are fully stabilized in the presence of a dinucleotide and a ribotriphosphate by the formation of the first phosphodiester bond. Once initiated, elongation with the wheat germ enzyme is stimulated by the presence of sarkosyl, an ionic detergent.

Materials and methods.

- Preparation of SV 40 DNA : African green monkey kidney cells (HP8, a subclone of CV1) were infected at a multiplicity of 10^{-4} pfu/cell with plaque purified SV 40 virus. Superhelical SV 40 DNA was extracted by the method of Hirt[12] and purified by isopycnic centrifugation in CsCl/ethidium bromide gradients.

- Enzyme purification : Calf thymus RNA polymerase II was purified according to the method described by Hods and Blatti[13] and wheat germ RNA polymerase II as described by Jendrisak and Burgess[14].

- Conditions for incubation of RNA polymerase with SV 40 DNA : The incubation mixture for the calf thymus enzyme contained : 30 mM Tris HCl (pH 7.9), 50 mM $(NH_4)_2SO_4$, 2 mM $MnCl_2$ and 0.5 mM dithiothreitol. The incubation mixture for wheat germ RNA polymerase II contained : 20 mM Tris HCl (pH 7.9), 40 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 0.5 mM dithiothreitol. The following concentrations of ribotriphosphates : 0.2 mM GTP, CTP, ATP and 0.1 mM (^{3}H)UTP (100 µCi/µmol) were added when transcription was studied.

Results.

The stability of preformed binary complexes between RNA polymerase and DNA can be measured kinetically by analysing the fraction of active enzyme resisting the action of a polyanion, as a function of time. As we have already shown with the E.coli and the calf thymus II enzymes[15,16], the complexes formed with superhelical DNA are more stable than those formed with linear or relaxed DNA. Identical results were obtained with the wheat

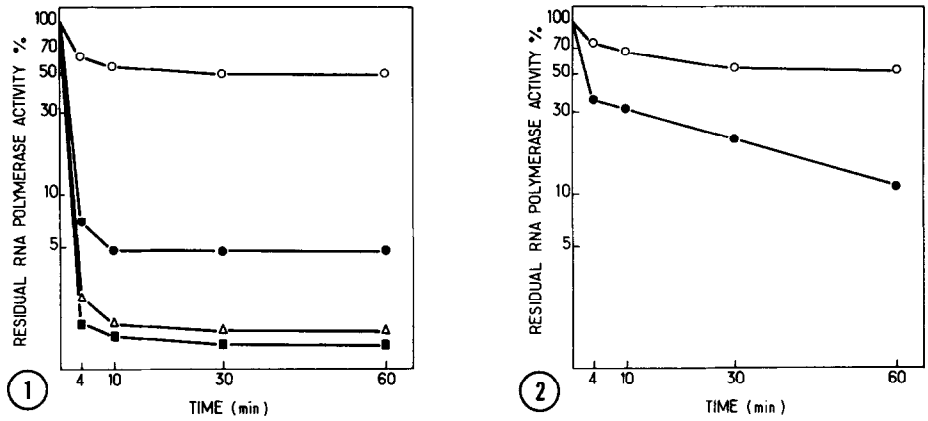


Figure 1. Stabilization by a dinucleotide and a ribotriphosphate of calf thymus RNA polymerase II - SV 40 DNA FI rapid start complexes.

RNA polymerase was incubated with SV 40 DNA FI (0.3 unit/1 µg) in the standard buffer in the absence -■-■-, or in the presence of ApA (0.4 mM) -Δ-Δ-, or ATP (0.1 mM) -●-●- or both -o-o- for 5 minutes at 37°C. Heparin was then added at a final concentration of 15 µg/ml and the incubation was continued at 0°C. At various intervals, the fraction of bound active enzyme was determined by addition of the 4 ribotriphosphates (^{3}H-UTP). After 3 minutes of RNA synthesis at 37°C, the acid-insoluble material was measured. The initial RNA polymerase activity (100%) was measured after 5 minutes of preincubation by simultaneous addition of heparin and ribotriphosphates.

Figure 2. Stabilization by a dinucleotide and a ribotriphosphate of wheat germ RNA polymerase II - SV 40 DNA FI rapid start complexes.

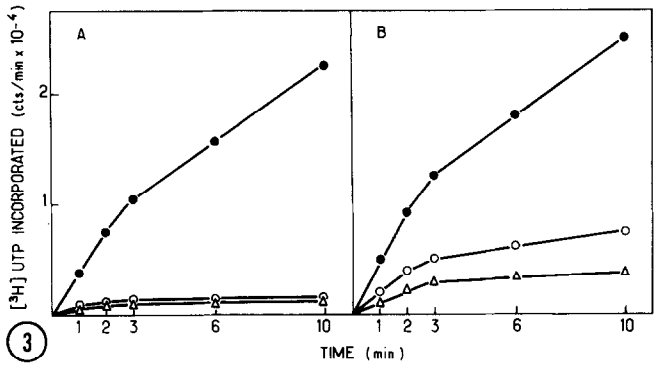
Wheat germ RNA polymerase was incubated with SV 40 DNA FI (0.3 unit/1 µg) as described in the legend of figure 1. The acid-insobule radioactivity was determined after 2,5 minutes transcription at 37°C. Preincubation without effectors -●-●-, or with ApA and ATP -o-o-.

germ RNA polymerase II[17,18]. As expected, supercoiled DNA is much better template for the three RNA polymerases. When measuring the stability of enzyme-superhelical DNA complexes, in the presence of heparin, the half life time observed with the E.coli enzyme was about 30 hours at 37°C whereas it was of the order to 30 seconds with the calf thymus enzyme[15]. When the dissociation rate of the calf thymus enzyme and FI DNA was measured at 0°C, less than 2% of activity remained after 4 minutes (Figure 1). The results given in Figure 2 show that the wheat germ RNA polymerase II exhibits a biphasic dissociation curve in the presence of

heparin : whereas 65% of the initial binary complexes dissociate in less than 4 min, the rest of the complexes dissociates with an half life time of about 30 minutes. The entire activity was sensitive to 0.1 µg/ml of α-amanitin confirming that we are dealing with enzyme II devoid of any contamination with RNA polymerase III.

Previous studies with the E.coli enzyme have shown that the concentration of ribotriphosphates required for initiation is higher than that necessary for elongation[19]. Dinucleotides complimentary to the DNA sequence at promoter sites stimulate specifically the binding of the enzyme to this site[20]. Furthermore, the formation of the first phosphodiester bond between the dinucleotide and the incoming ribotriphosphate further stabilizes the enzyme DNA complex [21]. A major initiation site for the E.coli RNA polymerase was mapped at position 0.17 of the SV 40 genome[22-24]. Since the RNA 5' sequence starts with pppApApA in this position, we decided to use the combination of ApA and ATP to assay for the formation of stable initiation complexes. When the rate of dissociation of non specific complexes formed by the E.coli enzyme was increased by the addition of 0.6 M KCl and incubation at 0°C, both ATP (5×10^{-6}M) and ApA (0.4 mM) were required for the stabilization of the initiation complexes (results not shown). When a similar experiment was performed with the calf thymus RNA polymerase II and FI SV 40 DNA, again, both ApA and ATP were required for the formation of stable initiation complexes (Figure 1). Similarly to these enzymes, about 50% of the wheat germ RNA polymerase II remains bound to the DNA in an heparin stable complex under these conditions (Figure 2). Electron microscopic observations of complexes stabilized with ApA and ATP confirmed that the E.coli enzyme binds preferentially to the promoter like site at 0.17 genome length[25]. However, both the calf thymus and the wheat germ enzymes did not show preferential binding at this site. These enzymes initiate with the same sequence as several other positions on the SV 40 genome[25].

The stable ternary complexes formed between the enzyme, the template and the nascent ribooligonucleotide permit elongation. In the presence of a polyanion like heparin, this elongation of the RNA chain is limited to only three minutes of synthesis at 26°C as shown in Figure 3-A, with the calf thymus



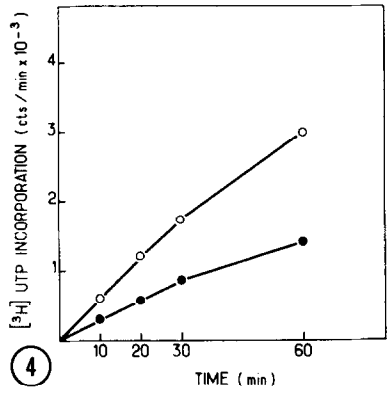


Figure 3. Effect of heparin and sarkosyl on in vitro transcription of superhelical SV 40 DNA by eucaryotic RNA polymerase II.

A.- Calf thymus RNA polymerase II.

B.- Wheat germ RNA polymerase II. (^{3}H) cRNA was synthesized in the standard buffer at a ratio of enzyme to DNA of 0.1 unit/1 µg at 26°C. Transcription was initiated by addition of the NTP and at various intervals samples were precipitated with 5% TCA, filtered on nitrocellulose membranes, dried and counted with liquid scintillation.

RNA synthesis without inhibitors -●-●-.

RNA synthesis in the presence of sarkosyl added at a final concentration of 0.2%, 5 seconds after synthesis initiation -o-o-.

RNA synthesis in the presence of heparin added at a final concentration of 15 µg/ml, 5 seconds after synthesis initiation -Δ-Δ-.

Figure 4. Transcriptional activity of wheat germ RNA polymerase II - SV 40 DNA FI stable complexes in the presence of sarkosyl.

Wheat germ RNA polymerase was preincubated with SV 40 DNA FI in the standard buffer for 5 minutes at 26°C. Heparin was then added to a final concentration of 15 µg/ml and the incubation continued at 0°C for 2 minutes. The reaction mixture was divided in two equal volumes, and RNA synthesis was measured at 26°C with the four ribotriphosphates -●-●-, or in the presence of ribotriphosphates and 0.2% sarkosyl -o-o-.

enzyme. On the contrary, the elongation with the wheat germ enzyme continues for up to 60 minutes, although with a reduced rate relative to the initial one (Figure 3-B and figure 4). Interestingly, with the wheat germ but not with the calf thymus enzyme, elongation of the transcript is stimulated by the addition of sarkosyl, an anionic detergent, as shown in Figure 3.

The elongation of RNA chains in heparin resistant binary complexes obtained after 2 minutes of dissociation at 0°C (see Figure 2) was also tested. As shown in Figure 4, the bound wheat germ polymerase molecules synthesize RNA linearly for 30 minutes at 26°C ; this elongation is stimulated about two fold by sarkosyl.

Discussion.

The results presented in this paper demonstrate several differences between the kinetic properties of two eucaryotic RNA polymerases II (B) from wheat germ and calf thymus. The former enzyme seems to be heterogeneous, a fraction of the activity dissociates from superhelical DNA with a half life time of 30 min, an intermediate value between the dissociation rate of calf thymus (τ = 30 sec) and the E.coli enzyme (τ = 30 hrs). It is possible that a fraction of the wheat germ enzyme lost during purification a σ like factor that is required for the stabilization of binary complexes. Alternatively, a fraction of the enzyme may have been subjected to partial proteolytic cleavage. The calf thymus enzyme may be similar to the deficient wheat germ enzyme. Another indication that a possible factor easily dissociable from the enzyme exists, is the change observed during purification in the requirement for bivalent cations. The enzyme after the DEAE-cellulose step functions better with Mg^{++} than with Mn^{++} whereas the reverse situation is observed after the phosphocellulose chromatography (results not shown). Similarly, Link and Richter[26] described a protein factor that changes the dependence of plant RNA polymerase II from Mn to Mg cations. Our results on the relative stability of the binary complexes formed with the wheat germ enzyme are in agreement with the studies of Seidman et al.[27] who detected stable complexes between this enzyme and adenovirus 2 DNA. Such complexes were also detected by comigration of this enzyme with supercoiled plasmid DNA on agarose gels[18].

Once ternary complexes were formed between the enzymes and the DNA, elongation with the wheat germ enzyme can be stimulated by the addition of sarkosyl. This situation is similar to the stability of endogeneous RNA polymerase bound to SV 40 or polyoma DNA, where elongation is observed in the presence of sarkosyl[28].

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