ON THE LIBERATION OF σ AND THE MOLECULAR WEIGHT OF *E. COLI* RNA POLYMERASE

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1. Introduction

In previous communications [1, 2] it was shown that RNA polymerase and the core enzyme lacking σ can be separated by polyacrylamide gel electrophoresis from the DNA–enzyme complex excited by deoxyribonuclease. This excised complex, which retains an amount of template resistant to DNase digestion of about 75 nucleotides [1], is shown, in the present work, to have a lower molecular weight than RNA polymerase due to the loss of the σ subunit. On the other hand, the detachment of σ during RNA synthesis appears to take place after the initiation process, when the nascent RNA chain reaches a minimum length.

2. Materials and methods

DNA-dependent RNA polymerase, core enzyme and σ subunit were prepared as already described [2]. Polynucleotide phosphorylase and aspartate transcarbamylase were gifts of Dr. M.N. Thang, and Dr. G. Hervé; the other proteins used were obtained commercially.

Deoxyribonuclease excision of the RNA polymerase–DNA complex: the mixture (450 μl) contained RNA polymerase or core enzyme, 80 μg, T_4 phage DNA 100 μg and standard salts [2]. After 5 min at 37° pancreatic deoxyribonuclease, 10 μg, was added, and the mixture was further incubated 5 min at 37°. To obtain the ternary *initiation complex,* the same incubation was carried out in the presence of ATP, UTP, CTP, 50 nmoles each. Molecular weight determination of RNA polymerase and the deoxyribonuclease excised complexes were performed following the method of Hedrick and Smith [3] : 50 μl aliquots of the above mixtures (or 10 to 20 μg of the proteins to be analysed) were layered on polyacrylamide gels of graded porosity, prepared at pH 8.3. Electrophoreses were performed at a constant current of 5 mA per gel with bromophenol blue as tracking dye. At the end of the run the gels were cut at the level of the dye and stained with coomassie brilliant blue and the migration of the protein band and the length of the gel were measured. The results are ex-

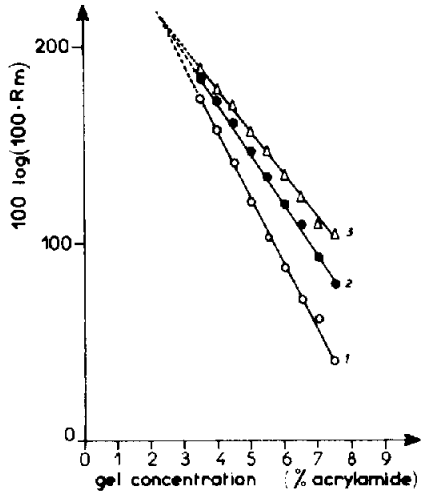

Fig. 1. Electrophoretic mobility of core enzyme [1], RNA polymerase [2] and deoxyribonuclease treated RNA polymerase–DNA complex [3]. Electrophoreses were carried outon polyacrylamide gels at different gel concentration and the migration of the protein bands was measured as described in Methods.

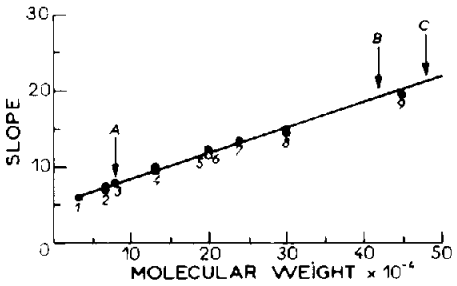

Fig. 2. Molecular weight estimation of RNA polymerase, σ subunit and template bound enzyme. Experiments similar to that shown in fig. 1 were carried out for a number of marker proteins, and the slope of the plots measured. The protein markers are designated by numbers: (1) pepsin, (2) albumin monomer, (3) alkaline phosphatase, (4) albumin dimer, (5) polynucleotide phosphorylase, (6) albumin trimer, (7) pyruvate kinase , (8) aspartate transcarbamylase, (9) ferritin. Are designated by the arrow: (A) sigma subunit; (B) DNase treated enzyme–DNA complex, enzyme–tRNA complex, DNase treated initiation complex (see Methods): (C) RNA polymerase.

pressed as the ratio of protein migration to dye migration (R_m). In some cases the migration of different molecules could be measured in the same gel, for example the RNA polymerase–tRNA complex and the liberated σ subunit, the DNase excised binary complex and the residual RNA polymerase. The liberation of σ was quantitated by scanning the gels with the Joyce and Loebl Chromoscan.

3. Results and discussion

To estimate the molecular weights of RNA polymerase, the core enzyme and the template bound enzyme excised by DNase, their electrophoretic mobility was measured at different gel concentration as described by Hedrick and Smith [3]. The plots obtained in fig. 1, are no parallel lines and intersect at low gel concentration. This indicates that the main difference between these three molecules is their molecular weight. From the calibration curve given in fig. 2, the molecular weight of RNA polymerase is estimated as 480,000 ± 30,000 daltons, whereas the core enzyme is oligomeric and runs as a protein heavier than 800,000 daltons; the excised binary complex is estimated as 420,000 ± 30,000 daltons and the σ subunit 80,000 ± 5,000 daltons. The DNase excited initiation complex corresponding to RNA polymerase incubated with T_4 DNA and ATP, UTP, CTP, behaves also as a material of 420,000 daltons, interestingly enough just as the DNase treated complex between core enzyme and T_4 DNA.

The low molecular weight of the DNase excised complexes, compared to RNA polymerase, suggested that they could be incomplete enzyme lacking the σ subunit whose molecular weight equals approximately the difference in molecular weight observed. This observation lead us to study the loss of σ by RNA polymerase under various conditions (fig. 3). To follow more easily the release of σ, the gels were heavily loaded with protein although this causes some enzyme molecules to aggregate as can be seen in the upper part of the gels. First of all, electrophoresis of RNA polymerase alone and of the T_4 DNA–enzyme complex (fig. 3A and B) does not result in a band migrating at the level of the purified free σ subunit (fig. 3C). Thus, as already reported by Krakow [4] the mere binding of RNA polymerase to native DNA does not displace the σ subunit. In contrast, the liberation of σ occurs after binding of RNA polymerase to tRNA, as shown for (A). Vinelandii RNA polymerase [4] (fig. 3H), and after DNase treatment of the T_4 DNA–RNA polymerase complex (fig. 3D). This loss of σ explains the difference in molecular weight found between RNA polymerase and the DNase treated complexes. The mechanism of this DNase promoted release of σ is not yet clarified. Several possibilities can be considered. Even though free σ subunit does not bind to DNA [2], it is conceivable that the initiation factor recognizes a sequence of nucleotide of the template whose hydrolysis induces the release of σ. Alternatively, the hydrolysis of the DNA might give rise to single stranded oligonucleotides which bind RNA polymerase and release σ as observed with denatured DNA [4].

In the same experiment the liberation of σ was followed without addition of DNase during the early steps of RNA synthesis. The release of the σ subunit during RNA synthesis, in the presence of the four nucleoside triphosphates (fig. 3E) was taken as control and estimated by scanning the gel. Fig. 3G shows that in absence of initiating nucleotides ATP and GTP, no liberation of σ subunit occurs. Initiation of short RNA chains in the presence of three nucleotides, ATP,

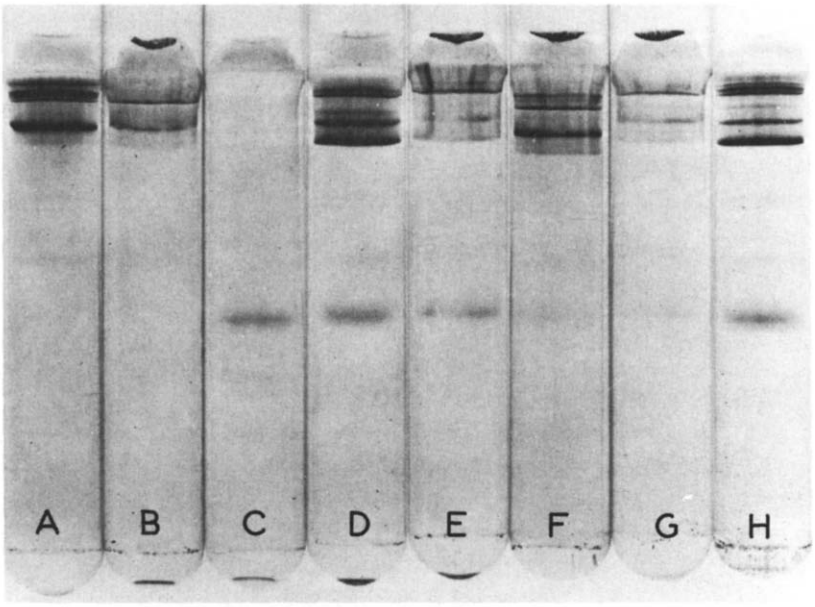

Fig. 3. Liberation of σ subunit at different steps of RNA synthesis as seen by polyacrylamide gel electrophoresis. (A) RNA polymerase (30 μg); (B) RNA polymerase plus T_4 DNA (20 μg) and standard salt 5 min at 37°; (C) sigma subunit (5 μg); (D) DNase treatment of RNA polymerase–T_4 DNA complex; (E) RNA polymerase, T_4 DNA, ATP, UTP, CTP, GTP (10^{-4} M each) and salts, 5 min at 37°; (F) as E without GTP; (G) as E without ATP and GTP; (H) RNA polymerase plus tRNA (5 μg). Electrophoreses were performed in 6.5 percent acrylamide gel. At the end of the run, the protein bands were stained and the liberation of σ was estimated by scanning the absorbancy of the gel at 550 nm.

UTP and CTP causes the appearance of small amounts of σ subunit, in the range of 5 to 15% of the amount liberated in the control. Thus it can be suspected that the liberation of the initiation factor σ does not occur during the initiation process itself, but after the RNA chains have reached a certain length. Evidence that σ is not liberated during the formation of the first phosphodiester bond is provided by using the model of Krakow and Fronk [5]. These authors have shown that pyrophosphate exchange can occur with poly d(AT) as template, 5′-AMP as initiator nucleotide and UTP. Using their system, where only one phosphodiester bond can possibly be made, no σ liberation was observed (result not shown), whereas during poly r(AU) synthesis, σ was fully liberated, as already noted by Krakow [4]. Thus it seems that the RNA chain has to reach a minimum length before σ release can occur. This supports the hypothesis [5] that σ interacts with an RNA binding site on the polymerase and is pushed off when the nascent RNA becomes long enough. Another possibility is that RNA polymerase undergoes a conformational change inducing the release of σ when the nascent RNA can form a stable hybrid with the template.

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