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Subunit Composition of Bacillus subtilis RNA Polymerase During Transcription

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Summary

The subunit composition of RNA polymerase isolated from Bacillus subtilis was determined under conditions which allowed the formation of stable enzyme-DNA complexes and under conditions which permitted transcription. These investigations showed that the delta peptide was released upon formation of enzyme-DNA complexes whereas the sigma subunit was released during transcription.

DNA-dependent RNA polymerase of procaryotes consists of a complex of several peptides. The polymerase from Escherichia coli can be separated into two functional units: the core assembly which contains 5 peptides (β',β,2α,ω) and the sigma subunit (1). RNA polymerase isolated from Bacillus subtilis can be separated into three functional units: a core assembly similar to that obtained from the E. coli polymerase except that it contains 2 omega peptides (β',β,2α,ω_1,ω_2), a sigma subunit and a 21,000 molecular weight peptide (delta 2,3) which has no known analogy in the E. coli enzyme.

As in other procaryotic polymerases, the specificity of transcription by the B. subtilis polymerase is provided by the sigma subunit; thus, with SP82 DNA as a template, sigma directs the binding of the core assembly to "early promoters" (4). Previous studies (3) have revealed that transcriptional specificity is also influenced by the delta peptide. For example, this peptide affects strand selection when SP82 DNA is the template, it stimulates transcription from homologous templates and

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inhibits transcription from heterologous templates, it increases the dissociation of enzyme-DNA complexes, it increases the translatability of RNA synthesized in vitro and it stimulates the synthesis of larger RNA molecules. The first three effects suggest a role in promoter recognition whereas the latter two raise the possibility that delta functions in elongation.

Williamson and Doi (5) examined the binding of delta and sigma subunits to core polymerase and reported that sigma was displaced from the core assembly in the presence of an excess of delta. This finding was interpreted as indicating that core + delta formed specific complexes with DNA which were subsequently initiated by sigma in the presence of nucleotides. An alternative possibility is that the delta peptide binds to assemblies of core + sigma after the initial site selection and initiation events and acts to promote RNA elongation.

To distinguish between these alternatives, we have determined the subunit composition of RNA polymerase when it is complexed to DNA and when it is actively transcribing DNA. The results of these experiments, which are reported here, indicate that delta is released during the formation of stable complexes between SP82 DNA and polymerase and that the sigma subunit is released upon initiation of RNA synthesis.

Materials and Methods

Methods have been described for the growth of B. subtilis, for the preparation of phage DNA and RNA polymerase and for the isolation of purified polymerase subunits (3,4,6-8).

The technique of Bodier and Rossetti (9) was used to examine the subunit structure of RNA polymerase. To examine polymerase bound to DNA, 15-25 µg of enzyme was incubated with 100-150 µg of SP82 DNA in 0.6 ml of Buffer A (10 mM tris HCl, pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM dithiothreitol, 100 mM NaCl) for 10 min at 37°. To examine polymerase transcribing SP82 DNA, RNA synthesis was initiated by adding 0.7 mM (final concentration) of each nucleotide triphosphate to enzyme-DNA mixtures. After 4 min of incubation at 37°, heparin (30 µg/ml, final concentration) and actinomycin (1.5 µg/ml, final concentration) were added. The samples were layered on discontinuous glycerol gradients and centrifuged for 3 3/4 hours at room temperature (21-25°) at 229,000 x g in an International SB282 swinging bucket rotor. The discontinuous glycerol gradients were composed of a bottom layer of 5 mls of 60% glycerol in Buffer A and a top layer consisting of a 7 ml gradient of 10-20% glycerol in the same buffer.

The gradients were fractionated by piercing the bottom of the centrifuge tubes and collecting 0.6 ml fractions. Control gradients were assayed for the position of DNA by measuring absorbancy at 260 nm and for the position of free and DNA-bound RNA polymerase using the standard RNA polymerase assay (6). The positions of purified sigma and delta in control gradients were determined by gel electrophoresis of fractions as described below.

To analyze enzyme present in various fractions, the fractions were combined by three, yielding a total of 7 fractions per gradient. After addition of 5 µg of DNAase I (Worthington), the combined fractions from each gradient were dialyzed against 100 ml of 10 mM tris HCl, pH 7.9, 10 mM $MgCl_2$, 0.1 mM EDTA, 50 mM β-mercapto-2-ethanol, 5% glycerol, 50 µg/ml phenylmethylsulfonylfluoride, 70 µg/ml L-1-tosylamide-2-phenylethylchloromethylketone (both purchased from Sigma Co.) overnight at 4° C, then dialyzed against 1 liter of distilled water for 12-24 hours at 4° C and lyophilized to dryness. Dried samples were dissolved in 40 µl of sample buffer and electrophoresed on one-dimensional polyacrylamide slab gels as described by Hiatt and Whiteley (8). After staining with Coomassie blue, the individual lanes were cut out and scanned at 600 nm with a spectrophotometer equipped with a Gilford linear transport scanner. The areas under the peaks on the scans were measured using an Electronic Graphics Calculator (Numonics Corp.) and expressed as molar ratio relative to (2α).

Results

Subunit composition of RNA polymerase. The content of sigma and delta in purified preparations of RNA polymerase vary depending on the methods of enzyme isolation. For example, chromatography on DEAE-Sephadex removes delta specifically and chromatography on phosphocellulose removes both sigma and delta. In addition, sedimentation of the polymerase through 10-30% glycerol gradients containing 0.4 M NaCl, a procedure used in our method of isolating RNA polymerase, separates core assemblies with different quantities of sigma and delta peptides. Hence it has been difficult to determine a "characteristic" quantity of delta for this enzyme. The range of molar ratios for active polymerase preparations is from 0.5 to 1.7 delta peptide/2α (2α is used as a measure of intact core assembly). We have previously measured the stimulation of activity observed when increasing amounts of purified delta are added to enzymes lacking the delta peptide (3) and found that maximum activity was obtained at a delta/2α ratio of 2.0. The ratio of sigma/2α varies in our preparations from 0.3 to 0.8/2α. Similar variability has been reported for polymerases from other bacteria and it was suggested that the amount of sigma is related to the growth rate (10).

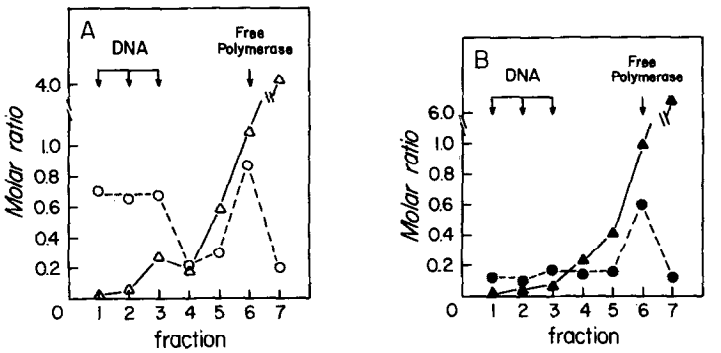

Fig. 1 Subunit compositon of RNA polymerase bound to DNA

RNA polymerase-DNA complexes were sedimented through glycerol gradients as described in the Materials and Methods before (panel A) or after (panel B) incubation allowing RNA chain initiation and elongation. The molar ratios of delta (triangles) and sigma (circles) to core polymerase was measured by scanning stained polyacrylamide gels of the gradient fractions, assuming 2α subunits/core assembly. The molecular weights assumed for the subunits were: delta, 21,000; α, 44,000 and sigma, 56,000. The indicated positions of DNA and free polymerase were determined in control gradients.

Composition of polymerase bound to SP82 DNA in the absence and presence of nucleotides. Fig. 1 shows the molar ratios of sigma and delta to core assembly in the fractions obtained from glycerol gradients under conditions which permit the formation of enzyme-DNA complexes and under conditions which allow elongation of RNA chains. As indicated, DNA was found predominantly in fractions 1 and 2. Separate experiments showed that free enzyme was detected in fraction 5, free delta peptide was found exclusively in fraction 7 and purified sigma was found in fractions 6 and 7. Thus, all of the enzyme subunits detected in fractions 1-3 must come from polymerase bound to DNA.

In the experiment shown in Fig. 1A, approximately 25% of the total enzyme was recovered in fractions 1-3 with most of the remainder in fractions 5 and 6. Fraction 4 routinely contained the smallest amount of polymerase and virtually no core assembly was found in fraction 7. In these experiments, it was necessary to add an excess of enzyme so

that sufficient protein could be recovered for analysis by scanning. When nucleotides were included in the reaction mixture (Fig. 1B), slightly more enzyme (30-50%) was recovered complexed to DNA but a substantial portion still sedimented as free polymerase.

The enzyme applied to the gradient in the experiment shown in Fig. 1 contained a molar ratio of sigma/2α of 0.6 and a molar ratio of delta/2α of 1.1. The molar ratios for these peptides in the polymerase bound to DNA in the absence of nucleotides (Fig. 1A, fractions 1-3) indicated a high content of sigma and effectively no delta. Polymerase which sedimented as free enzyme contained larger amounts of sigma and delta whereas fraction 7 contained a large amount of delta peptide.

The addition of nucleotides prior to sedimentation did not affect the amount of delta found in enzyme bound to DNA. As seen in Fig. 1B, the content of delta in fractions 1-3 was effectively zero as in the preceding experiment and a large amount of delta was found in fraction 7. However, addition of nucleotides prior to sedimentation did affect the molar ratio of the sigma subunit recovered in the DNA-bound enzyme: fractions 1-3 contained a greatly diminished amount of sigma. These observations indicate that the delta subunit was released when the enzyme-DNA complex was formed and that the sigma subunit was released, as in the *E. coli* polymerase (11), during transcription.

Discussion

The present experiments show that the sigma subunit remained bound to the core during the formation of stable enzyme-DNA complexes and was released upon initiation whereas delta was released upon formation of complexes with DNA which were stable enough to withstand the conditions of centrifugation employed in these experiments. The formation of stable enzyme-DNA complexes and their isolation by sedimentation depends on a number of as yet not fully defined variables. It is not clear why delta is released from complexes isolated by sedimentation whereas

polymerase which has been purified by chromatography on DNA-cellulose retains delta. This suggests that the sedimentation procedure may affect the association of enzyme subunits complexed to DNA or that sedimentation selects certain kinds of complexes. If the latter explanation is correct, the difference in association before and after delta release may be subtle and subject to experimental conditions. It should be noted that in some experiments (3 out of 25), delta was found with sedimented enzyme-DNA complexes.

Previous studies on the effect of delta on the specificity of transcription suggested that delta increases the ability of polymerase to recognize promotor regions. This could account for the delta-directed stimulation of polymerase activity on homologous templates and selective inhibition of activity on heterologous templates. Furthermore, the stimulation of RNA chain elongation and translatability of RNA synthesized *in vitro* (3) could be the result of more efficient binding of polymerase to promoter regions. The present data suggest that delta has its effect on a step in enzyme-DNA interaction which preceeds the formation of tight binding complexes since it is absent from these complexes and from ones elongating RNA chains. A model for delta action proposed by Williamson and Doi (5) suggested that sigma and delta do not bind to the same core assembly and that core + delta assemblies for the initial complexes with DNA which are then acted on by sigma after the release of delta. However, it seems unlikely that delta itself would specify recognition of initiation sites since it is conserved during changes in initiation specificity which occur following infection of *B. subtilis* with SP82 (3,4,7,8) or SP01 (2,12). It seems reasonable to assume that delta and sigma act in concert in the initial stages of DNA site selection. The release of delta from the enzyme-DNA complexes signals a definable stage in the initiation reaction which should be of interest for further study.

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