TRYPSIN MODIFICATION OF DNA-DEPENDENT RNA POLYMERASE OF E COLI B

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SUMMARY

(1) The effects and course of tryptic digestion of RNA polymerase subunits are described. (2) Proteolysis in the absence of DNA causes a straight-forward loss of polymerising activity in both core and holoenzyme. However, proteolysis of a DNA - core or holoenzyme complex results in a considerable increase in specific activity. Digestion of the DNA- core enzyme complex can be limited to a cleavage of only the β subunit. (3) The β subunit is cleaved approximately one third along its length into two fragments β_1 = 124,000 and β_2 = 52,000 molecular weight. (4) The σ subunit was particularly labile to proteolysis whereas β' and α subunits were comparatively resistant. (5) Extensive degradation in the presence of DNA results in a still partially active complex.

INTRODUCTION

E.coli RNA polymerase core enzyme (EC 2.7.7.6) consists of four subunits β, β', α_2, with a mean molecular weight for $\beta\beta'$ of 170,000, and 42,000 for α subunits (1). An additional subunit σ, of molecular weight 95,000, is involved in the recognition of specific transcription sites on DNA (2). *In vivo* limited proteolytic cleavage of the RNA polymerase isolated during sporulation of *B.subtilis* (3) and *B.thuringiensis* (4) has been linked with the changes in specificity for the DNA template.

In this communication we describe the structural effects and functional consequences of limited proteolysis of *E.coli* RNA polymerase subunits. The effect of bound DNA on the proteolysis is also examined.

MATERIALS AND METHODS

E.coli RNA polymerase core enzyme was isolated as described previously (5). Holoenzyme containing 0.33 moles sigma per mole of core enzyme was isolated (6) with double-stranded DNA affinity chromatography (7) replacing phosphocellulose chromatography and all subsequent steps.

RNA polymerase was assayed by monitoring the incorporation of [^{14}C] AMP into acid insoluble material (8). Assays contained 5 mg/ml bovine serum albumen where indicated. Analytical polyacrylamide gel electrophoresis was carried out in 5% gels containing 0.1% sodium dodecyl sulphate (9) or 3% gels containing 8M urea (10). Separation of β from β' is possible on urea gels whereas separation on gels containing 0.1% SDS is incomplete due to the similarity in the molecular weight of the subunits. The molecular weights of fragments produced by proteolysis were estimated (9) using *E.coli* RNA polymerase subunits ($\beta\beta'$, σ, α) as markers (1).

For proteolysis up to 1.5 mg/ml of RNA polymerase in 0.05M KCl, 0.05M Tris-(or triethanolamine) HCl, pH 8, 0.01M $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA, were incubated at 37°with $^1/_{100}$ - $^1/_{10000}$ parts by weight of trypsin (Sigma Chemical Co.) per part RNA polymerase. The amount of trypsin and time of incubation depended upon the amount of digestion required. Samples were removed for assays which were supplemented with bovine serum albumen. For

electrophoretic analysis samples were removed and added to an equal volume of 10.5M urea: 1% SDS: 0.1M dithiothreitol (5:1:1 by vol. - for gels containing urea, SDS was omitted from this mixture) and applied directly to the gels.

Single stranded sonicated DNA was prepared by the sonication of cooled 2mM native DNA for 6 x ten second bursts followed by boiling for 10 min. The denatured DNA had 28% hyperchromicity at λ 260. RNA polymerase and DNA were complexed as described in the legends to the figures.

RESULTS

Proteolysis of the RNA Polymerase-DNA Complex

Limited proteolysis in the presence of DNA leads to a marked increase in activity in both core and holoenzyme (Figs. 1 &2). This increase in specific activity is associated with cleavage of

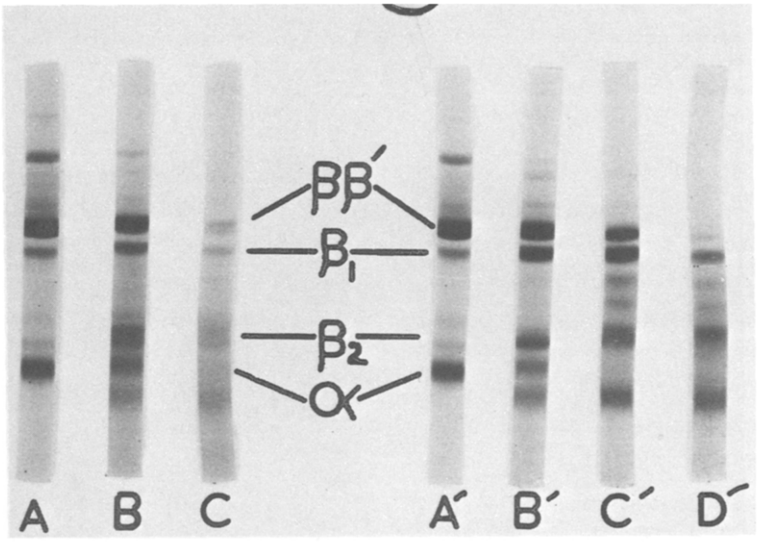

Figure 1

Proteolysis of Core Enzyme With and Without DNA at Various Trypsin Levels

A 0.6 mg/ml solution of core enzyme in the above buffer, with or without 0.5 mM DNA was incubated for 1 hour at room temperature.), $^1/_{10000}$, $^1/_{1000}$ or $^1/_{200}$ parts (w/w) of trypsin/core enzyme were added as indicated below, and the incubation continued for 1 hour. 60 µg denatured enzyme were applied to 3% polyacrylamide-SDS gels and aliquots removed for assay. A = -DNA, -TRYPSIN (100% activity); B = -DNA + $^1/_{10000}$ (w/w) trypsin (113% activity); C = -DNA + $^1/_{1000}$ (w/w) trypsin (14% activity). A′ = +DNA - Trypsin (100% activity); B′ = +DNA + $^1/_{10000}$ (w/w) trypsin (85% activity); D′ = PIP = +DNA + $^1/_{200}$ (w/w) trypsin (28% activity).

the β subunit into two new fragments, β_1 (M.W. = 124,000) and β_2 (M.W. = 52,000). That β is the parent subunit of β_1 and β_2 is demonstrated in Figs. 3 & 4, where at this level of proteolytic treatment there is quantitative cleavage of β without any other subunit being affected. β_2 fails to appear on urea gels. The cleavage of β is more rapid in the presence of DNA than in its absence.

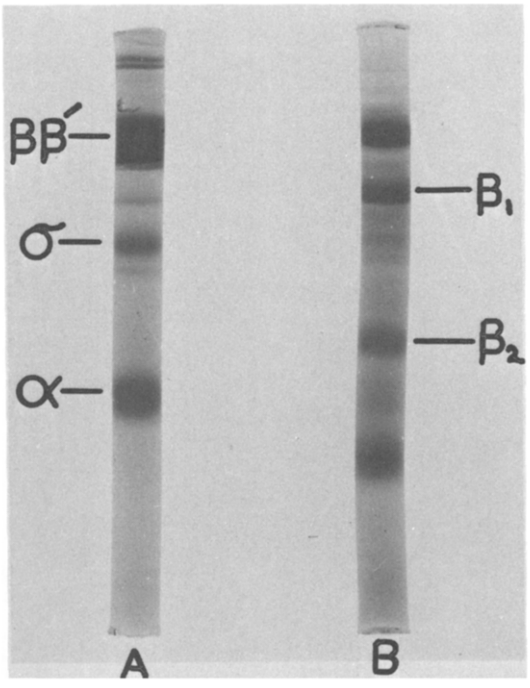

Figure 2

Proteolysis of Holoenzyme in the Presence of DNA

0.5 mg/ml holoenzyme in the above buffer + 1 mM DNA was incubated at 1 hour at room temperature. An aliquot was assayed (100% activity) and 50 µg of enzyme applied to A. $^{1}/_{1000}$ (w/w) trypsin was added and incubation continued for 1 hour. An aliquot was assayed (114% activity) and 50 µg of enzyme applied to B. 5% polyacrylamide-SDS gels were run for 2½ hours.

As a consequence of this enhanced susceptibility, it is possible to stop the digestion when the β subunit alone has been cleaved. From a consideration of the β_1 β_2 fragment molecular weights this would appear to be the product of a 'single-hit' corresponding to the disruption of a single peptide bond. The sigma subunit of the holoenzyme - DNA complex is digested away before the cleavage of the β subunit is complete (Fig. 2). Extensive proteolysis by prolonged incubation of core or holoenzyme in the presence of DNA with high levels of trypsin leads to the formation of complexes containing only β_1, β_2 and small molecular weight material. This protease incubated polymerase* still maintains considerable polymerising function (Fig. 2, D').

Proteolysis of RNA Polymerase in the Absence of DNA

Proteolytic digestion in the absence of DNA leads to extensive loss of polymerising activity in both core and holoenzyme (Fig. 1 & 5). It is clear that the attack occurs simultaneously on different subunits, though at differing rates. It is possible to interpret the pattern of breakdown as a result of the more clearly defined events occurring during the digestion of the DNA

* Protease Incubated Polymerase = (PIP)

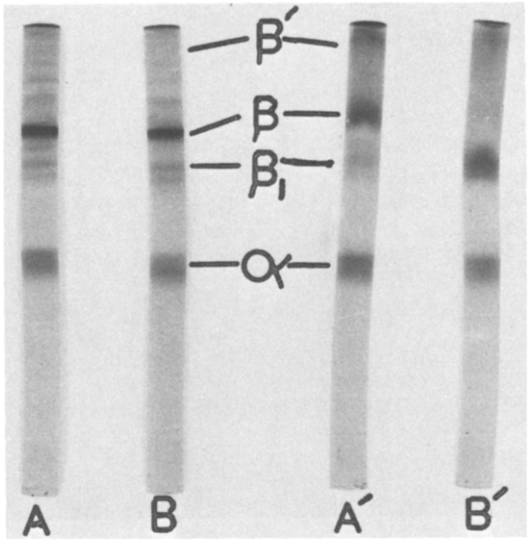

Figure 3

Comparison of Tryptic Cleavage of Core Enzyme, Complexed or Uncomplexed with DNA, using Urea Gels

To a 1 mg/ml solution of core enzyme in the above buffer, with or without 0.7 mM DNA, $^{1}/_{1000}$ parts (w/w) trypsin/core enzyme were added or omitted. The mixture was then incubated for 2 mins. at 37° C and 20 µg denatured RNA polymerase applied to each 3% urea polyacrylamide gel as follows: A = -DNA -trypsin; B = -DNA +trypsin; A′ = +DNA - trypsin; B′ = +DNA +trypsin.

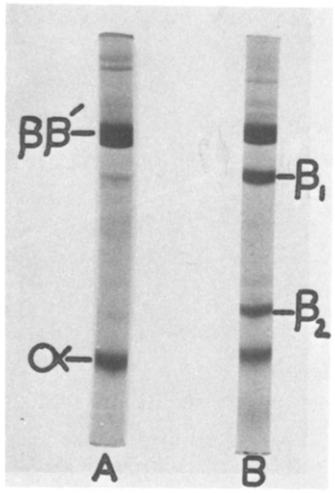

Figure 4

Formation of β_1 and β_2 by Proteolysis of a DNA-Core Enzyme Complex

To a 0.5 mg/ml core enzyme solution in the above buffer, with or without 0.28 mM sonicated DNA, $^{1}/_{1000}$ parts (w/w) of trypsin/core enzyme were added or added or omitted. The mixtures were then incubated for 10 minutes at 37° C and 40 µg of denatured enzyme applied to each 5% polyacrylamide-SDS gel as follows: A = -DNA -trypsin; B = +DNA +trypsin.

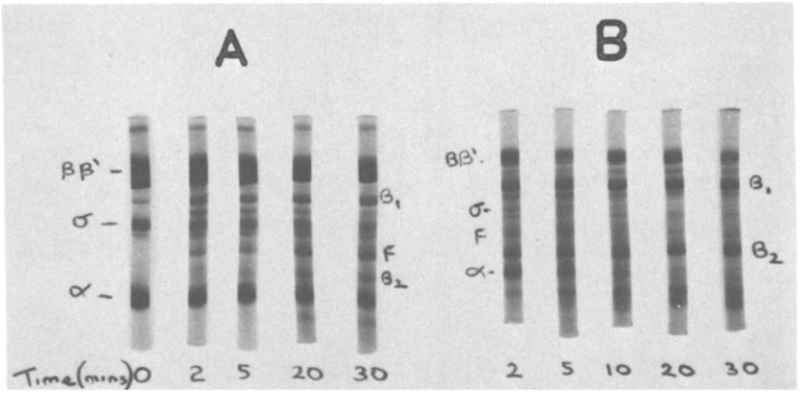

Figure 5

Temporal Sequence of Holoenzyme Subunit Cleavage in the Absence of DNA
A 1 mg/ml solution of holoenzyme in the above buffer was incubated at 37° C for the times shown, with A $^{1}/_{10000}$ and B $^{1}/_{1000}$ parts (w/w) trypsin/holoenzyme. 100 µg of denatured enzyme was applied to each 5% polyacrylamide-SDS gel. Aliquots were removed for assay. After 30 minutes incubation the RNA polymerase activity levels had fallen to A = 81% and B = 14% of the unproteolysed controls.

complexed polymerase. The observed temporal sequence of subunit cleavage can be seen from Fig. 5. There is a rapid formation of the two fragments β_1 and β_2 with simultaneous rapid digestion and elimination of sigma. The α and β' sub-units are broken down more slowly into lower molecular weight material. F is a transient labile fragment also found in the digestion of core enzyme and therefore not a sigma breakdown product.

DISCUSSION

The results show that the RNA polymerase - DNA complex can be cleaved without loss of activity. Of added significance is the finding that the β subunit shows an enhanced susceptibility to cleavage when the polymerase is complexed to DNA. We have previously reported (5) independent evidence that DNA imposes a constraint on the structural motility of the protein. As a result a discrete new species is formed which, suprisingly, has a higher specific activity than the native precursor. β_1 is closely related in size to polypeptides formed by *in vivo* cleavage of *B.subtilis* (M.W.= 110,000) and *B.thuringiensis* (M.W. = 130,000). Occasionally RNA polymerase is isolated from *E.coli* containing small amounts of β_1 and β_2. Furthermore, the sigma subunit is highly labile to proteolytic digestion - the mechanism which was postulated as the cause of the DNA template affinity changes occurring during sporulation in *B.subtilis* (3). There have been numerous reports of low sigma content in preparations of RNA polymerase holoenzyme, a possible reason being proteolytic removal of the sigma subunit.. The extensively protease incubated polymerase (PIP), formed only in the presence of DNA, is significant in that it still maintains some activity. Experiments are currently in progress to isolate and characterise the active (PIP) particle.

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