# DIFFERENTIAL ACTION OF TRYPSIN ON THE $\beta'$ SUBUNIT OF DNA-DEPENDENT RNA POLYMERASE FROM *E.COLI*

J.P. LECOCQ *

*Laboratoire de Génétique, Université libre de Bruxelles*
*67, rue des Chevaux, 1640 Rhode St Genèse, Belgium*

Received 7 May 1971
Revised version received 8 June 1971

## 1. Introduction

Enzymatic hydrolysis of proteins may inform us about their native structure and the functions of their different subunits [1]. We used this approach in a study of DNA-dependent RNA polymerase from *E. coli.* This enzyme can be isolated in two forms: a core or minimal enzyme (catalytic unit) whose subunit structure is $\beta', \beta, 2\alpha$ and a holoenzyme containing an additional subunit, the $\sigma$ factor, necessary for specific initiation [2, 3]. Zillig et al. [4] have shown the role of the $\beta'$ chain in the primary binding on the DNA template and that of $\beta$ in initiation of RNA synthesis.

The experiments described here show: (1) that incubation of RNA polymerase with trypsin results in a parallel decrease of polymerase activity and DNA binding; (2) that $\beta'$ is by far the most sensitive of the subunits; (3) that the loss of enzymatic activity is prevented by preincubation with DNA.

## 2. Materials and methods

DNA-dependent RNA polymerase was partially purified according to Burgess [5].

A sample of highly purified RNA polymerase, kindly provided by Dr. R. Burgess, was used in the late phase of this work.

$^{14}C$ labelled λ DNA was prepared in this laboratory by J. De Lafonteyne.

* I.R.S.I.A. fellow.

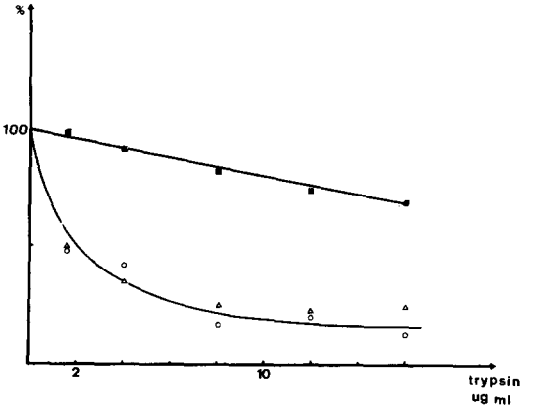


Fig. 1. RNA polymerase activity (○) and DNA binding capacity (△) following 15 min incubation with different concentrations of trypsin. Transcriptional activity was also measured on samples of RNA polymerase preincubated with calf thymus DNA (■).

Polyacrylamide gel electrophoresis was performed in gels containing either 5% acrylamide, 0.1% sodium dodecyl sulphate, and 0.1 M $Na_2HPO_4$ pH 7.2 or 7.5% acrylamide, 8 M urea pH 8.7.

Tryptic hydrolysis was carried out at 30° in buffer containing tris-HCl 0.04 M pH 8, $MgCl_2$ $8 \times 10^{-3}$ M, $MnCl_2$ $10^{-3}$ M, β-mercaptoethanol $5 \times 10^{-3}$ M. Digestion was arrested by adding Soybean trypsin inhibitor 15 min later at a concentration of 250 μg/ml and the mixture was incubated an additional 15 min at 30°.

Other samples of RNA polymerase were preincubated with DNA for 5 min at 30° in the same buffer.

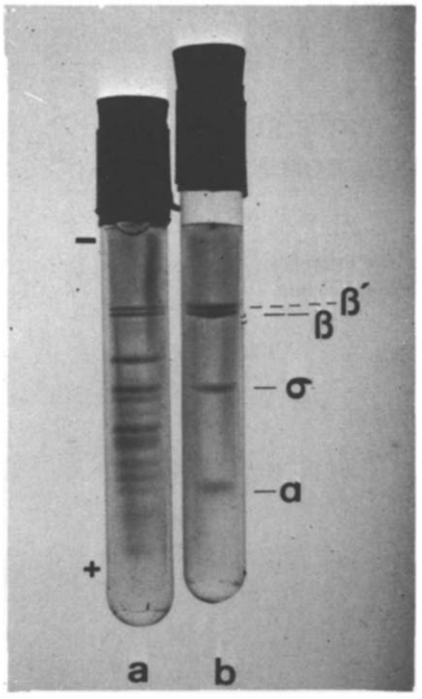


Fig. 2. Control run with extremely purified (99%) RNA polymerase kindly provided by Dr. R. Burgess.

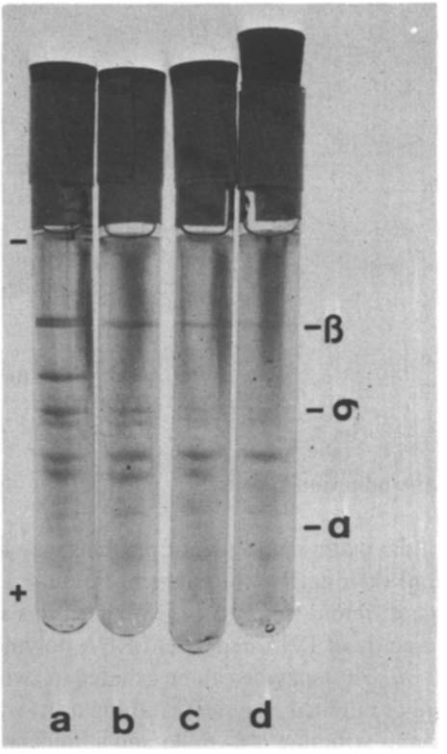


Fig. 3. SDS polyacrylamide gel electrophoresis of partially purified RNA polymerase untreated (a) or incubated with 1.6 μg/ml (b), 8 μg/ml (c) or 16 μg/ml (d) trypsin.

For the RNA polymerase assay, calf thymus DNA (where necessary) and nucleosides triphosphates were added at a concentration of 80 μg/ml and 0.4 mM, respectively, together with UTP $^{3}H$ (5 μCi/μM).

Binding of RNA polymerase to DNA was measured by the conventional nitrocellulose membrane techniques [6].

## 3. Results and discussion

Transcriptional activity and DNA binding capacity of RNA polymerase were studied following 15 min incubation with different concentrations of trypsin. Clearly, tryptic hydrolysis of RNA polymerase results in parallel losses of its transcriptional activity and DNA binding capacity. Preincubation of the RNA polymerase with DNA exerts a marked protective effect on this degradation (fig. 1).

The effect of trypsin digestion on the different subunits of RNA polymerase was followed on SDS and urea polyacrylamide gels. Incubation with low concentration of the proteolytic enzyme results in the disappearance of one of the bands of the β' β doublet; the other subunits are obviously less affected (figs. 2 and 3).

That the trypsin sensitive subunit is β' was made clear by running together a mixture of intact and partially hydrolysed RNA polymerase. The possibility that β' is modified to a form which runs like β and that it is β which is destroyed, is made very unlikely by the identity of the patterns observed in two completely different gel systems (figs. 3 and 4).

Our results are thus consistent with the views that: (1) as shown by Zillig et al., β' is responsible for the binding of enzyme on template; (2) the effect of trypsin on the enzymatic activity of RNA polymerase is primarily due to the loss of DNA binding capacity, which is itself a result of the destruction of β' subunit.

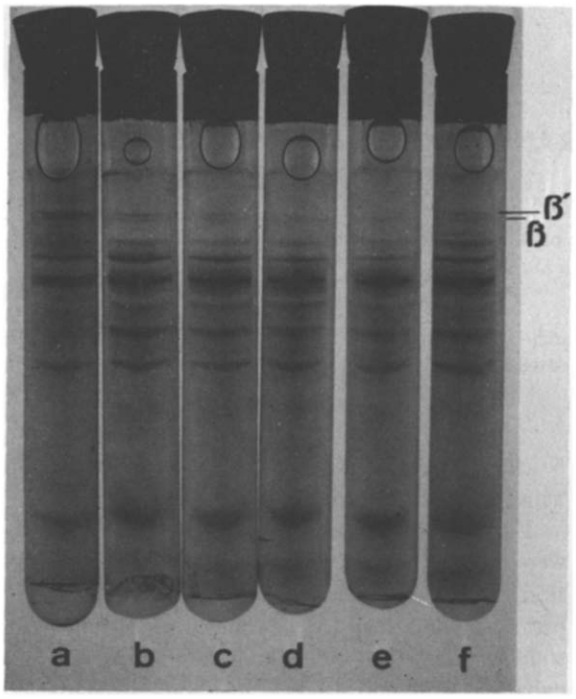


Fig. 4. Urea polyacrylamide gel electrophoresis of partially purified RNA polymerase untreated (a) incubated with 0.2 μg/ml (b), 0.5 μg/ml (c), 1 μg/ml (d), or 2 μg/ml trypsin (e), and of a mixture of a part (1/3) of "a" plus "e" (f).

*Note:* After this paper was written, our attention was drawn to a paper of R.D. Khesin et al. [7] who showed increased stability of RNA polymerase to various agents (including trypsin) in the presence of DNA.

**Acknowledgements**

The author thanks Prof. R. Thomas and Dr. A. Bollen for helpful discusssions. This work was carried out under a Euratom-ULB contract 007-61-10 ABIB, a Belgian Government-ULB contract and with the support of the "Fonds de la Recherche Fondamentale Collective".

**References**

[1] F.N. Chang and J.G. Flaks, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1321.
[2] R.R. Burgess, A.A. Travers, J.J. Dunn and E.K.F. Bautz, Nature 221 (1969) 43.
[3] R.R. Burgess and A.A. Travers, Federation Proc. 29 (1970) 1164.
[4] W. Zillig, K. Zechel, D. Rabussay, M. Schachner, V.S. Sethi, P. Palm, A. Heil and W. Seifert, Cold Spring Harbor Symp. Quant. Biol. (1970) 47.
[5] R.R. Burgess, J. Biol. Chem. 244 (1969) 6160.
[6] O.W. Jones and P. Berg, J. Mol. Biol. 22 (1966) 199.
[7] R.B. Khesin, O.B. Astaurova, M.F. Shemyakin, S.G. Kamzonova and V.F. Manyakov, Mol. Biol. 1 (1967) 736.