Functionally important site in the vicinity of the amino-terminus of the *Escherichia coli* RNA polymerase β subunit

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We have analyzed the interaction of monoclonal antibodies against *Escherichia coli* RNA polymerase with products of its limited proteolysis. Two major proteolytic fragments of molecular masses 107 and 43 kDa originate as a result of a single cleavage in the vicinity of the 980th amino acid residue. Anti-β subunit monoclonal antibody PYN-2 inhibiting RNA polymerase activity at the stage of RNA elongation reacts with an epitope located between the amino-terminus and the 50th amino acid residue of the β subunit. DNA sequencing has shown that the RNA polymerase mutation *rpoB22* converts the Gln(1111) codon of the β subunit gene into the amber codon. An epitope for the monoclonal antibody PYN-6 was located between the major site of proteolytic cleavage and Gln(1111) of the β subunit.

RNA polymerase *Monoclonal antibody* *Limited proteolysis* *Epitope mapping* *Amber mutation*

1. INTRODUCTION

Monoclonal antibodies may serve as powerful tools for probing the structure and functions of complex multimeric proteins such as DNA-dependent RNA polymerases. Previously we have obtained a collection of monoclonal antibodies against RNA polymerase from *Escherichia coli* [1]. Only a few antibodies against β and β′ subunits inhibited RNA polymerase activity. Here we describe experiments on the interaction of monoclonal antibodies with products of a limited proteolysis of RNA polymerase and with an amber fragment of the β subunit. As a result of these experiments 2 epitopes were localized in the β subunit and some proteolytic fragments of the β subunit were identified.

2. MATERIALS AND METHODS

Core RNA polymerase and its subunits were obtained as described [2]. Core RNA polymerase was cleaved by trypsin (Spofa, Czechoslovakia) in a buffer containing 0.01 M Tris, pH 7.5, 0.15 M NaCl, 5% glycerol at room temperature for 1.5 h, the RNA polymerase:trypsin ratio being 1000:1 (w/w). Products of proteolysis were separated in an SDS-polyacrylamide slab gel according to Laemmli [3] and transferred to nitrocellulose (Millipore HA) as described in [4]. Antibody binding was visualized by the ELISA technique as described [1]. The *E. coli* strain with an RNA polymerase amber mutation *rpoB22* was described in [5]. Cells were grown in LB medium, harvested at a density of 4×10^8 cells/ml, and disrupted in an ultrasonic disintegrator as in [5]. DNA sequence analysis was performed by the method of Maxam and Gilbert as described in [6].

3. RESULTS AND DISCUSSION

Limited proteolysis of RNA polymerase from *E. coli* has been used extensively to study the structure and functions of the enzyme [7–14]. Two major metastable breakdown products of 107 and 43 kDa are easily recognized (fig.1). At first it was concluded that these fragments arose as a result of a single cleavage of the β subunit [8]. Later it was

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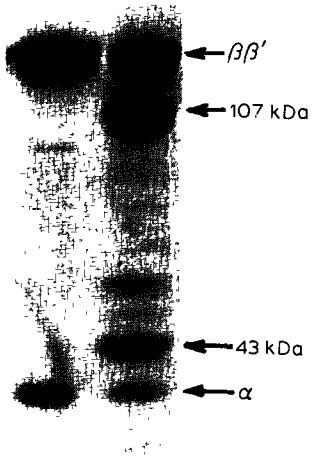

Fig.1. Limited proteolysis of core RNA polymerase. RNA polymerase preparations were subjected to electrophoresis on 10% SDS-polyacrylamide gel. Lanes: 1, untreated; 2, treated with trypsin.

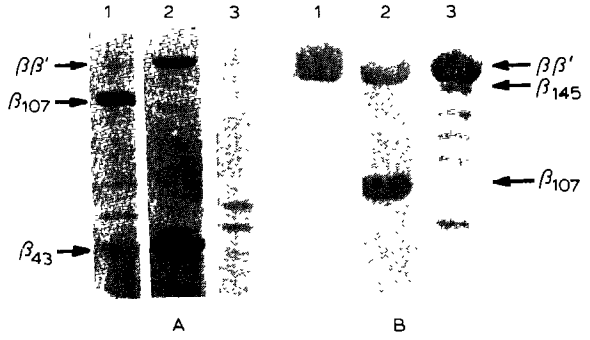

Fig.2. Binding of monoclonal antibodies to proteolytic fragments of RNA polymerase. Core RNA polymerase preparations treated with trypsin were subjected to electrophoresis on SDS-polyacrylamide gel (A, 10%; B, 5%), transferred to nitrocellulose and reacted with monoclonal antibodies. Lanes: 1, PYN-2 (anti β); 2, PYN-6 (anti β); 3, PYN-1 (anti β').

claimed that these fragments originated from the β' subunit [10] or from both the β and β' subunits [11]. These conflicting conclusions are most probably due to the lack of resolution of the β and β' subunits and their slightly shortened fragments on the SDS-polyacrylamide gel. To resolve these contradictions we studied the interaction of RNA polymerase proteolysis products and monoclonal antibodies against β and β' subunits.

Fig.2A shows that an anti-β subunit monoclonal antibody PYN-2 reacts with the 107 kDa fragment but not with the 43 kDa fragment. Another anti-β subunit antibody PYN-6 reacts with the 43 kDa fragment but not with the 107 kDa fragment. Anti-β' subunit antibodies PYN-1 (fig.2) and PYN-3 (not shown) do not react with either the 107 kDa or 43 kDa fragment. These data clearly show that both the 107 and 43 kDa fragments originate from the β subunit. Therefore we named the fragments β_{107} and β_{43}.

It should be noted that our data on the origin of the β_{107} and β_{43} fragments make it necessary to reconsider some of the inferences drawn from DNA-RNA polymerase cross-linking experiments by Park et al. [15,16] who believed that β_{107} and β_{43} originate from the β' subunit.

We tested 5 different anti-β monoclonal antibodies. Each reacted with either β_{107} or β_{43}. These results indicate that β_{43} is not a product of proteolytic degradation of β_{107}. Since the sum of the molecular masses of β_{107} and β_{43} equals that of the β subunit (150 kDa) one can conclude that β_{107} and β_{43} arise after a single cleavage of the β subunit, as first suggested in [8].

The next step was to locate the cleavage site. Fig.2B shows that the β_{43}-specific antibody PYN-6 reacts with 4 minor RNA polymerase fragments larger than β_{107} (the largest of these fragments was named β_{145} according to its molecular mass). The β_{107}-specific antibody PYN-2 reacts with none of these fragments including β_{145}. This means that the PYN-2 antibody reacts with a terminal segment of the β subunit which is missing in β_{145}. To determine which terminal fragment is missing from β_{145}, we reacted the PYN-2 antibody with a fragment of the β subunit found in extracts of the RNA polymerase amber mutant RpoB22 [5]. DNA sequencing of the mutant β subunit gene has shown that the *rpoB22* mutation is a GC-AT transition which converts the Gln(1111) codon to the amber codon (details of mutant DNA cloning and sequencing will be published elsewhere). This corresponds to an amber fragment of 124.4 kDa. Fig.2B shows that the PYN-2 antibody reacts with a fragment of 125 kDa present in RpoB22 cell extracts. The coincidence of the predicted and observed molecular masses indicates that the 125 kDa fragment comes from termination on codon 1111 and contains an intact amino-terminus of the β subunit. One can conclude that it is the amino-terminus of the β subunit which is cleaved off in β_{145}, while the PYN-2 antibody reacts with

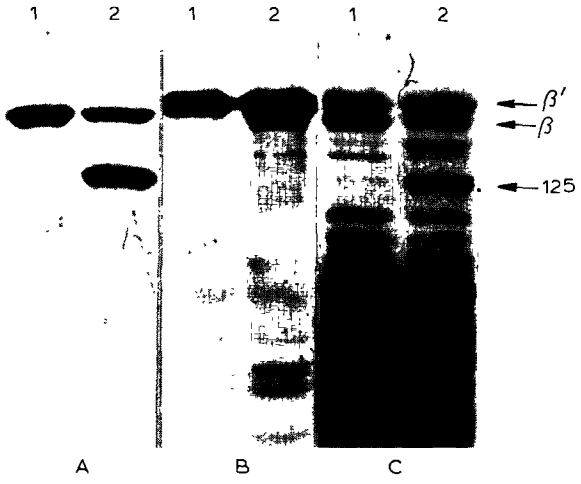

Fig.3. Binding of monoclonal antibodies to subunits of RNA polymerase and to the RpoB22 125 kDa amber fragment. Extracts of wild-type (1) and RpoB22 mutant (2) cells were subjected to electrophoresis on 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and reacted with monoclonal antibodies. A, reacted with anti-β antibodies PYN-2; B, reacted with anti-β' antibodies PYN-1; C, stained with amido black.

the amino-terminal region of the β subunit. The corresponding epitope is located somewhere between the 1st and approximately the 50th residue of the β subunit.

Since the PYN-2 antibody inhibits RNA polymerase at the stage of RNA elongation [1] we conclude that the amino-terminal segment of the β subunit is involved in RNA elongation.

Localization of the epitope for the PYN-2 antibody shows that β_{107} contains amino-terminal sequences of the β subunit while β_{43} carries its carboxy-terminal sequences. Therefore we conclude that the site of the proteolytic cleavage generating β_{107} and β_{43} is located in the vicinity of the 980th amino acid residue of the β subunit. One can speculate that in native RNA polymerase that region of the β subunit forms a free loop which is highly sensitive to proteolysis.

The anti-β_{43} antibody PYN-6 was shown to react with the RpoB22 amber fragment. This places the epitope for the PYN-6 antibody in the overlapping region of the 2 fragments, i.e. between the 980th and 1111th residues. The epitope may be localized with greater precision using amber fragments shorter than that of RpoB22 (like those described in [17]) and of a deletion mutant with shortened β [18].

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