PROTEOLYTIC CLEAVAGE OF DNA POLYMERASE FROM *ESCHERICHIA COLI* B INTO AN EXONUCLEASE UNIT AND A POLYMERASE UNIT

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1. Introduction

DNA polymerase from *E. coli* consists of a single polypeptide chain with a molecular weight of 109,000 [1]. The ezyme catalyzes the extension of polydeoxyribonucleotides from the 3′-OH end group, the exchange of inorganic pyrophosphate with the two terminal phosphate groups in deoxyribonucleoside triphosphates, and an exonucleolytic degradation of polydeoxyribotides from both the 3′-hydroxyl and the 5′-phosphate end groups [2, 3].

It has recently been shown in this laboratory that treatment of DNA polymerase from *E. coli* B with the proteolytic enzyme subtilisin (type Carslberg) under the assay conditions used results in an increase of the polymerase activity and a concomitant decrease of the exonuclease activity to a level of few percent of the initial value. The resulting DNA polymerase has been isolated and a molecular weight of about 70,000 has been estimated from gel filtration experiments. This enzyme has been found to be almost completely devoid of exonuclease activity, assayed with ^{3}H-poly-d(A-T) [4]. Experiments leading to very similar results have recently been independently performed by Brutlag et al. [5].

We wish to report on the treatment of native DNA polymerase with subtilisin under conditions which lead to the cleavage of the enzyme into two separate subunits, one which is associated with exonuclease activity and the other with polymerase activity.

2. Experimental

The materials and methods were the same as previously described [4], except that ^{3}H-poly-d(A-T) had a four fold higher specific activity (250 μCi μmole^{-1}). The enzyme assays were also as previously described except that the DNA polymerase assay was performed in potassium phosphate buffer pH 7.4 (30 mM) in absence of potassium chloride.

3. Results

It has previously been found that the exonuclease activity, in contrast to the polymerase activity, decreases rapidly on incubation of native DNA polymerase with subtilisin (type Carlsberg) [4]. It now appears that under somewhat altered conditions this differential susceptibility to subtilisin becomes less pronounced (fig. 1 curves 1). Under these conditions the effect of the presence of DNA in the incubation mixture has been studied. It may be seen from fig. 1 that the presence of DNA to a great extent protects both the exonuclease activity and the polymerase activity against deterioration due to subtilisin treatment. Subtilisin treatment in the presence of DNA even results in an increase in exonuclease activity followed by a small decrease, while there is an immediate decrease in its activity in the absence of DNA. The polymerase activity increases both in the absence and in the presence of DNA. In the latter case, the activity remains high while in the absence of DNA, it decreases after about

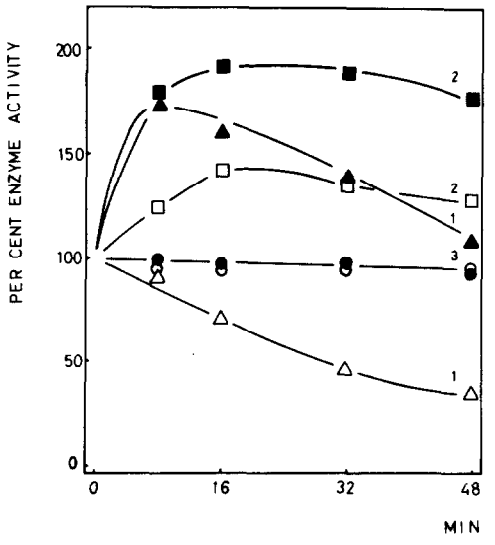

Fig. 1. Influence of DNA on the effect of subtilisin on native DNA polymerase. Native DNA polymerase (170 μg ml^{-1}) was incubated at 37°C in potassium phosphate buffer (0.10 M, pH 6.5) containing mercaptoethanol (0.01 M), ammonium sulphate (30 mM), glycerol (0.39 M) and bovine albumin (113 μg ml^{-1}). To tube 1 (curves 1) subtilisin, type Carlsberg, (0.7 μg ml^{-1}) was added. To tube 2 (curves 2) subtilisin (0.7 μg ml^{-1}) and DNA (120 μg ml^{-1}) were added. To tube 3 (curve 3) DNA (120 μg ml^{-1}) were added. At time intervals indicated, samples of 10 μl were transferred to 90 μl were transferred to 90 μl solutions of bovine plasma albumin (30 mg ml^{-1}). In the case of the samples from tube 1, the solution of bovine plasma albumin also contained DNA (12 μg ml^{-1}). Of these dilutions, 30 μl were assayed for polymerase activity and 50 μl for exonuclease activity. Open symbols: exonuclease activity. Closed symbols: DNA polymerase activity.

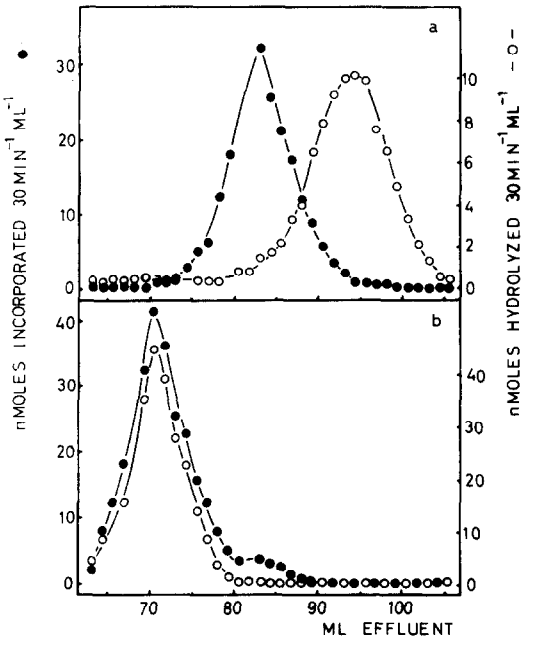

Fig. 2a. Gel filtration chromatography of subtilisin plus DNA treated native DNA polymerase. After incubation for 48 min, 525 μl of the reaction mixture from tube 2 of the experiment described in fig. 1 were cooled in ice and 1 M potassium phosphate buffer pH 6.5 was added to a final concentration of 0.2 M. This solution was passed through a column of DEAE-Sephadex (3 X 0.5 cm) equilibrated with 0.2 M potassium phosphate buffer. The column was eluted with the same buffer at a rate of about 60 μl min^{-1}. Fractions of 5 drops were collected. Fractions 4, 5, 6 and 7 were pooled; these contained about 70 percent of the polymerase activity and of the exonuclease activity. To 500 μl of this solution, a solution of avolbumin (9 mg, 60 μl) and blue dextran (4 mg) were added. This solution was passed into a column of Sephadex G-200 (1.5 X 81 cm). The column was the same as previously used and was operated in the same way except that fractions of 1240 μl were collected [4]. Of each fraction, 30 μl was assayed for polymerase activity and for exonuclease activity, respectively. The overall yield of the two activities was about 84 percent.

Fig. 2b. Gel filtration chromatography of native untreated DNA polymerase. To 500 μl of buffer (0.1 M ammonium sulphate, 0.1 M potasaium phosphate pH 7.0, 0.01 M mercaptoethanol) native enzyme (20 μl, 56 μg), ovalbumin (9 mg), muscle aldolase (5 mg) and blue dextran 200 (5 mg) were added. This solution was operated and the fractions assayed as described above.

8 min. The activities of both enzymes are almost completely unchanged in the presence of DNA alone.

It has previously been observed that the modification of the native polymerase by subtilisin to the 70,000 molecular weight species is accompanied by an increase in polymerase activity [4]. The increase in polymerase activity now observed in the presence of both DNA and subtilisin suggested, therefore, that the native polymerase in this case had also been modified by subtilisin to the 70,000 molecular weight species. In this case the remaining exonuclease activity might be located either in the same molecule as the modified polymerase or on a separate protein with a molecular weight of about 40,000 or less formed by cleavage of the native polymerase. In order to distinguish between these possibilities, the native polymerase was analyzed by gel filtration chromatography after incubation with subtilisin and DNA. Before

chromatography, the DNA in the incubation mixture was removed by treatment with DEAE-Sephadex. The effectiveness of this treatment was confirmed by the complete dependency of the polymerase activity of the resulting solution on the addition of DNA to the assay mixture. Analysis of the gel filtration chromatogram showed the presence of a single polymerase activity peak (elution volume 83 ml) and a single exonuclease activity peak (elution volume 94 ml) completely separated from each other (fig. 2a). From the calibration of the column with marker proteins as previously published, the molecular weights were estimated from the elution volumes according to Andrews [6] to be about 70,000 for the polymerase unit and about 35,000 for the exonucelase unit.

Gel filtration chromatography of untreated native DNA polymerase (fig. 2b) showed a single polymerase activity peak, with a shoulder at an elution volume of 83 ml. The polymerase activity peak coincided with a single symmetrical exonuclease activity peak. The elution volume of the peaks was 71 ml, identical with that previously found.

It may be concluded that native DNA polymerase, under specific conditions, may be split by proteolysis into two subunits with molecular weights of approximately 70,000 and 35,000. Under the assay conditions used, the larger molecule is associated with DNA polymerase activity and no exonuclease activity while the smaller one is associated with exonuclease activity and no polymerase activity. In the presence of specific substrates, Brutlag et al. [5] have isolated a modified DNA polymerase associated with 3′→5′nuclease activity but devoid of 5′→3′ nuclease activity. It is possible that the modified polymerase isolated in this laboratory has similar properties; in this case, the exonuclease assay used shows only 5′→3′ and not 3′→5′ nuclease activity.

4. Discussion

We conclude that an exonuclease site and a polymerase site of the native DNA polymerase are associated with different parts of the polypeptide chain and they may be separated from each other as catalytic active units by proteolysis under specific conditions. Thus the native DNA polymerase may be regarded as a true bifunctional enzyme.

The sum of the estimated moelcular weights of the two isolated enzymatic active units is also in agreement with the possibility that they are formed by cleavage of the native enzyme by hydrolysis of one or more peptide bonds. If the two units, like the native enzyme [1], both consist of a single polypeptide chain, the two parts of the native enzyme corresponding to the two units may be folded into separate tertiary structures. The two folded parts may be connected by a short peptide chain. In the presence of DNA one or more adjacent peptide bonds in this chain may be particularly susceptible to proteolytic attack. In the absence of DNA, peptide bonds important for catalytic activity are also susceptible to proteolysis. A conformation of this type for the native enzyme would considerably deviate from that of a sphere. It has previously been found that the elution volume of the native enzyme from a gel filtration column is smaller than expected for a spheric protein with a molecular weight of 109,000 [4]. The above proposed conformation of the native molecule is in agreement with this finding.

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References

[1] T.M.Jovin, P.T.Englund and L.L.Bertsch, J. Biol. Chem. 244 (1969) 2996.
[2] R.P.Klett, A.Cerami and E.R.Reich, Proc. Natl. Acad. Sci. U.S. 60 (1968) 943.
[3] M.P.Deutscher and A.Kornberg, J. Biol. Chem. 244 (1969) 3029.
[4] H.Klenow and I.Henningsen, Proc. Natl. Acad. Sci. U.S., in press.
[5] D.Brutlag, M.R.Atkinson, P.Setlow and A.Kornberg, Biochem. Biophys. Res. Commun., in press.
[6] P.Andrews, Biochem. J. 91 (1964) 222.