ATP-Stimulated Polymerase Activity Involving DNA Polymerase I and a *rec*B-Dependent Factor in Extracts of *Escherichia coli* Cells

Juhani Eerik Syväoja

Department of Biochemistry, University of Oulu, SF-90570 Oulu, Finland

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ATP-stimulated DNA polymerase activity involving DNA polymerase I has been found to be present in cell extracts from wild type and *rec*C mutant strains of *Escherichia coli,* but not in extracts from *rec*B strain. The activity has been separated from *rec*BC DNase by DEAE-cellulose ion exchange. It is suggested that *rec*B-dependent factor is involved in the ATP-stimulation of polymerase. Evidence is provided that this stimulation may be due to the interaction of *rec*B-dependent factor with DNA polymerase I.

In studies on ATP-dependent covalent closing of hydrogen-bonded lambda DNA rings in extracts of *Escherichia coli* it was observed that of the numerous mutants studied, this closing activity was low or absent in extracts of *lop*8*lig*4, *pol*A1 and *rec*B21 strains.[1,2] Normal levels of activity were found in extracts of *rec*C22 strain.[3] These findings may be interpreted to support the involvement of DNA ligase (EC 6.5.1.2), DNA polymerase I (EC 2.7.7.7) and *rec*B protein in the ATP-requiring ligation process. *rec*B and *rec*C genes code proteins for *rec*BC enzyme (EC 3.1.11.5).[4] Recently, evidence has been presented that a third subunit coded by *rec*D may be an essential component of this enzyme.[5] The enzyme has multiple catalytic activities *in vitro.* It is an ATP-dependent exonuclease acting on double and single strand DNA,[6] an ATP-stimulated endonuclease acting on single strand DNA,[7] a DNA helicase[8,9] and a DNA-dependent ATPase.[6] The subunit coded by the *rec*B gene,[10] possibly together with the subunit coded by *rec*D gene,[5] is responsible for the ATPase activity, while the *rec*C protein appears to have no enzymatic activity.[10]

The requirement of DNA polymerase I and a form of *rec*B protein for ligation is difficult to explain in terms of the known functions of these enzymes. While looking for forms of DNA polymerase I that might be involved in ATP-dependent ligation, a DNA polymerase activity which was stimulated by ATP was found in extracts of *E. coli,* and evidence for the requirement of at least DNA polymerase I and *rec*B or *rec*D protein for this activity was also obtained.

Experimental procedure

Materials. The following *E. coli* K12 strains were used: C600 and 1200, which is deficient in endonuclease I and RNase I activity,[11] were obtained from Dr. D. Court, and JC6722 *rec*B21 and JC 5489 *rec*C22, which both lack *rec*BC DNase activity,[12] were gifts from Dr. A. Clark. The mutations in these strains are located in different subunits of the *rec*BC enzyme. Strains W3110 *pol*A1, which is deficient in DNA polymerase I activity,[13] and W3110 thy$^-$, which is a thymine requiring mutant, were donated by Dr. H. Echols. Nucleoside triphosphates and DNA polymerase I were purchased from Boehringer (Mannheim), adenosine and thymidine from Fluka (Buchs) and radioactive [Me-^{3}H]thymidine and [Me-^{3}H]thymidine triphosphate from The Radiochemical Centre (Amersham). DNA polymerase I was from Boehringer (Mannheim).

*Enzyme assays. rec*BC DNase was assayed according to Eichler and Lehman[14] in the presence of 0.3 mM ATP. *E. coli* [^{3}H]DNA was used as the substrate. Labelled DNA was purified as described by Marmur[15] from strain W3110 thy$^-$, which was grown at 37 °C with constant aeration in 1 l of MM9 medium supplemented with 2 μg ml^{-1} of thymidine, 250 mg of adenosine and 200

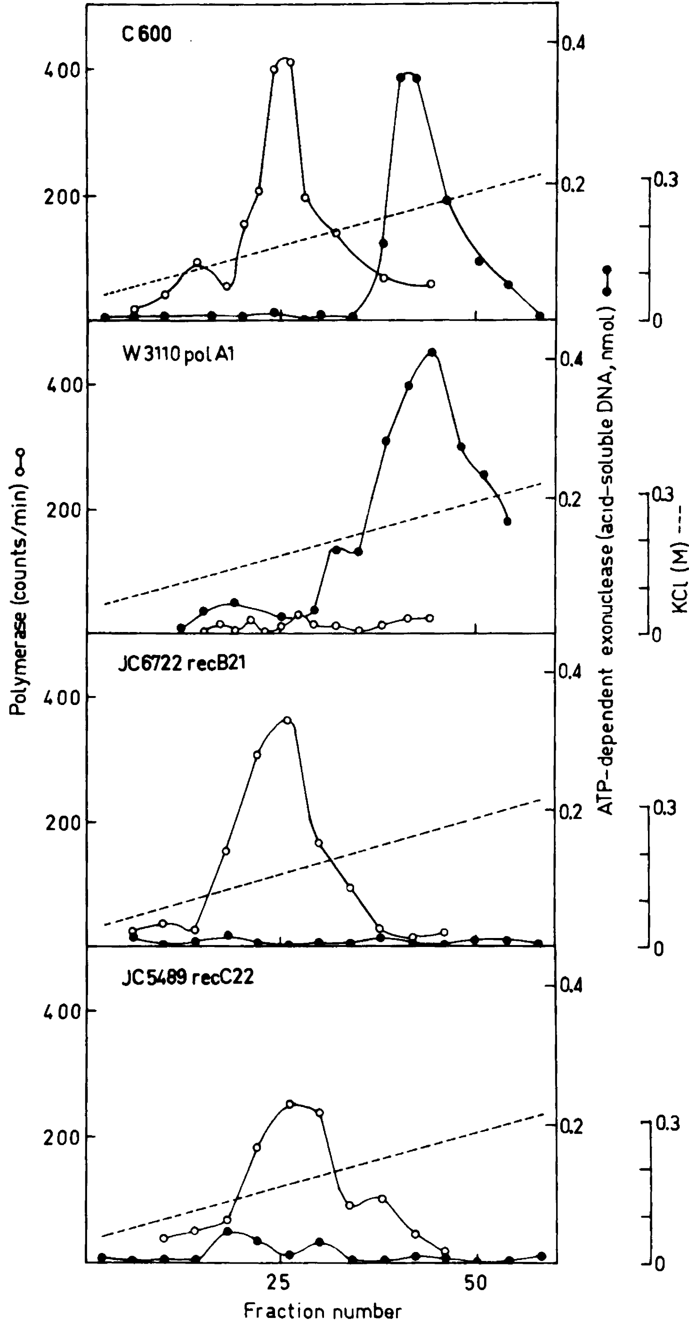

Fig. 1. Separation of DNA polymerase and ATP-dependent exonuclease activities of *pol*A1, *rec*B21 and *rec*C22 mutant extracts of *E. coli* in a DEAE-cellulose column. Strain C600 was used as a control.

μCi of [Me-^{3}H]thymidine until the absorbance at 590 nm of the culture was 1. The specific activity of the labelled DNA was about 1–5×10^3 cpm $nmol^{-1}$.

The reaction mixture for the assay of DNA polymerase I has been described by Hendler *et al.*,[16] and it contained 70 mM KCl and 0.3 μCi of [Me-^{3}H]thymidine triphosphate, together with native thymus DNA (Serva) as the template-primer. When ATP was present, its concentration was 1 mM. Following the incubation, DNA was precipitated, washed and solubilized according to the procedure of Lehman *et al.*,[17] and the radioactivity measured in a liquid scintillation counter.

Preparation of cell extract and DEAE-cellulose chromatography. The bacteria were grown in a medium containing 1.5 % tryptone, 1 % yeast extract and 0.5 % NaCl with constant aeration at 37 °C. Cells from 1 l of culture (about 2×10^9 cells ml^{-1}) were suspended in 0.5 ml of buffer A [20 mM Tris/HCl (pH 7.5), 1 mM dithiothreitol, 0.1 mM EDTA] and ruptured by sonication in short pulses in an MSE sonicator at an amplitude of 7 μ for 3 min. The lysate was diluted with 30 ml of buffer A and clarified by centrifugation at 25 000×g for 15 min at 4 °C. Solid ammonium sulfate was added over 20 min with constant stirring to a concentration corresponding to 50 % saturation. After stirring for a further 20 min, the precipitate was collected by centrifugation as described previously, dissolved in 2 ml of buffer A and dialyzed overnight against the same buffer.

The dialyzed ammonium sulfate precipitate containing 27 mg of protein was applied to a column of DEAE-cellulose (Whatman DE52, 0.64 cm^2×8 cm), which was equilibrated with buffer B [20 mM Tris/HCl (pH 7.5), 30 % (*v*/*v*) glycerol, 1 mM dithiothreitol, 0.1 mM EDTA]. After washing with 30 ml of 50 mM KCl dissolved in buffer B, the proteins were eluted with 120 ml of a linear salt gradient of 50–300 mM KCl in buffer B. Fractions of 2 ml were collected. Glycerol was added to the fractions to a concentration of 50 % (*v*/*v*) and they were stored at −20 °C.

The protein content of the extracts was determined according to Lowry *et al.*[18]

Results and discussion

DNA polymerase activity eluting at a concentration of 0.16 M KCl was found in extracts of the *E. coli* control strain C600 and the mutant strains *rec*B21 and *rec*C22, but not in extracts of strain *pol*A1 (Fig. 1). This indicates that DNA polymerase I is at least present in this gradient peak. Extracts of strain *pol*A1 are known to have only 1 % of the polymerizing activity of wild-type cells.[13] C600 strain ATP-dependent nuclease activity eluting at a KCl concentration of 0.23 M was well-separated from this polymerase activity. This elution pattern was also observed for the enzyme from strain 1200, which is an endonuclease I^- and $RNase^-$ strain. The peak of ATP-dependent nuclease activity was absent for strains *rec*B21 and *rec*C22, and it therefore apparently is the *rec*BC nuclease that is absent in *rec*B and *rec*C extracts.[12] The nuclease assay employed in this investigation is the same as used for the assay of *rec*BC nuclease.[14]

The DNA polymerase activities eluted from DEAE-cellulose columns with 0.16 M KCl were about equal, regardless of the strain studied, except for the strain *pol*A1 that lacked the activity (Table 1). However, the polymerase activity for control and *rec*C22 strains was stimulated to the extent of 61–73 % by ATP, but showed 15 % inhibition for *rec*B21 strain (Table 1). Since polymerase activity of the purified DNA polymerase I is not stimulated by ATP in the presence of the four DNTPs,[19] it is possible that the protein coded by *rec*B gene and/or *rec*D gene is somehow responsible for this stimulation. One possible explanation for ATP-stimulated polymerization is a direct interaction of some *rec*B-dependent factor with DNA polymerase I in the presence of ATP. In fact, the behaviour observed on addition of

Table 1. Stimulation of the DNA polymerase fraction from a DEAE-cellulose column by ATP. The activity of the gradient fraction from *E. coli* C600 was taken as 100 %. ATP stimulation was measured for the most active fraction of the gradient.

Strain	Activity in the absence of ATP/%	Stimulation by ATP/%
C600	100	+65
1200	130	+61
W3110 *pol*A1	<5	–
JC5489 *rec*C22	71	+73
JC6722 *rec*B21	106	−15

Table 2. Effect of DNA polymerase I addition on ATP stimulation of DNA polymerase activity. Activity was assayed for the polymerase peak fraction of *E. coli* C600 extract. The amount of purified DNA polymerase I added to the assay mixture was 0.1 units.

Sample	Acid-insoluble radioactivity/cpm		
	ATP omitted	ATP added	Difference due to the stimulation
DEAE-fraction	1020	1680	660
DEAE-fraction + *polI*	2613	3304	691

purified DNA polymerase I to the reaction mixture seemed to support this notion. As can be seen in Table 2, no increase in stimulation by ATP was observed, although polymerase activity was increased as expected.

Although ATP is unable to stimulate DNA polymerase I activity *in vitro,* in permeabilized cells it has been shown to stimulate DNA repair synthesis assumed to be catalyzed by DNA polymerase I.[20] This may mean that the enzyme forms a complex with some other proteins in the cell. A crude complex of DNA polymerase I and *rec*BC enzyme has been reported previous.[16,21] The polymerase activity of complex isolated from cells of *rec*B21 strain is not, however, inhibited by ATP, but shows about 45 % stimulation instead. The activity described here may thus be attributable to a novel form of DNA polymerase I. A more thorough analysis of the molecular structure and catalytic properties of the enzyme found will be carried out after further purification.

References

1. Pyhtilä, M. J. *Suomen Kemistilehti B 46* (1973) 72.
2. Pyhtilä, M. J. and Syväoja, J. E. *Eur. J. Biochem. 112* (1980) 125.
3. Syväoja, J. E. and Pyhtilä, M. J. *FEBS Lett. 183* (1985) 383.
4. Dykstra, C. C., Prasher, D. and Kushner, S. R. *J. Bacteriol. 157* (1984) 21.
5. Amundsen, S. K., Taylor, A. F., Chaudhury, A. M. and Smith, G. R. *Proc. Natl. Acad. Sci. U.S.A. 83* (1986) 5558.
6. Wright, M., Buttin, G. and Hurwitz, J. *J. Biol. Chem. 246* (1971) 6543.
7. Goldmark, P. J. and Linn, S. *Proc. Natl. Acad. Sci. U.S.A. 67* (1970) 434.
8. Rosamond, J., Telander, K. M. and Linn, S, *J. Biol. Chem. 254* (1979) 8646.
9. Muskavitch, K. M. T. and Linn, S. *J. Biol. Chem. 257* (1982) 2641.
10. Hickson, I. D., Robson, C. N., Atkinson, K. E., Tomkinson, A. E. and Emmerson, P. T. *J. Biol. Chem. 260* (1985) 1224.
11. Dürwald, M. and Hoffmann-Berling, H. *J. Mol. Biol. 34* (1968) 331.
12. Barbour, S. D. and Clark, A. J. *Proc. Natl. Acad. Sci. U.S.A. 65* (1970) 955.
13. Lehman, J. R. and Chien, J. R. *J. Biol. Chem. 248* (1973) 7717.
14. Eichler, D. and Lehman, I. R. *J. Biol. Chem. 252* (1977) 499.
15. Marmur, J. In: Colowick, S. P. and Kaplan, N. O., Eds, *Methods in Enzymology,* Academic Press, New York 1963, Vol. 6, p. 726.
16. Hendler, R. W., Pereida, M. and Scharff, R. *Proc. Natl. Acad. Sci. U.S.A. 72.* (1975) 2099.
17. Lehman, I. R., Bessman, M. J., Simms, E. S. and Kornberg, A. *J. Biol. Chem. 233* (1958) 163.
18. Lowry, O. H., Rosenbrough, N. I., Fan, A. L. and Randall, R. J. *J. Biol. Chem. 193* (1951) 265.
19. Spasokukotskaja, T., Staub, M., Sasvári-Szekely, M. and Antoni, F. *Biochim. Biophys. Acta 656* (1981) 140.
20. Gärtner, C., Waldstein, E. A. and Hagen, V. *Biochim. Biophys. Acta 67* (1980) 247.
21. Scharff, R., Hanson, M. A. and Hendler, R. W. *Biochim. Biophys. Acta 739* (1983) 265.

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