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

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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 recC mutant strains of Escherichia coli, but not in extracts from recB strain. The activity has been separated from recBC DNase by DEAE-cellulose ion exchange. It is suggested that recB-dependent factor is involved in the ATP-stimulation of polymerase. Evidence is provided that this stimulation may be due to the interaction of recB-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 lopBlig4, polA1 and recB21 strains.1,2 Normal levels of activity were found in extracts of recC22 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 recB protein in the ATP-requiring ligation process. recB and recC genes code proteins for recBC enzyme (EC 3.1.11.5).4 Recently, evidence has been presented that a third subunit coded by recD 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 helicase8,9 and a DNA-dependent ATPase.6 The subunit coded by the recB gene,10 possibly together with the subunit coded by recD gene,5 is responsible for the ATPase activity, while the recC protein appears to have no enzymatic activity.10

The requirement of DNA polymerase I and a form of recB 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 recB or recD 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 recB21 and JC 5489 recC22, which both lack recBC DNase activity,12 were gifts from Dr. A. Clark. The mutations in these strains are located in different subunits of the recBC enzyme. Strains W3110 polA1, 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-3H]thymidine and [Me-3H]thymidine triphosphate from The Radiochemical Centre (Amersham). DNA polymerase I was from Boehringer (Mannheim).
Enzyme assays. recBC DNase was assayed according to Eichler and Lehman\textsuperscript{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\textsuperscript{15} from strain W3110 thy\(^{-}\), which was grown at 37\(^\circ\)C with constant aeration in 1 l of MM9 medium supplemented with 2 \(\mu\)g ml\(^{-1}\) of thymidine, 250 mg of adenosine and 200

Fig. 1. Separation of DNA polymerase and ATP-dependent exonuclease activities of polA1, recB21 and recC22 mutant extracts of *E. coli* in a DEAE-cellulose column. Strain C600 was used as a control.

333
µCi of [Me-3H]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., and it contained 70 mM KCl and 0.3 µCi of [Me-3H]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., 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 x 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 x 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.

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 recB21 and recC22, but not in extracts of strain polA1 (Fig. 1). This indicates that DNA polymerase I is at least present in this gradient peak. Extracts of strain polA1 are known to have only 1% of the polymerizing activity of wild-type cells. 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 1⁻ and RNase⁻ strain. The peak of ATP-dependent nuclease activity was absent for strains recB21 and recC22, and it therefore apparently is the recBC nuclease that is absent in recB and recC extracts. The nuclease assay employed in this investigation is the same as used for the assay of recBC nuclease.

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 polA1 that lacked the activity (Table 1). However, the polymerase activity for control and recC22 strains was stimulated to the extent of 61–73% by ATP, but showed 15% inhibition for recB21 strain (Table 1). Since polymerase activity of the purified DNA polymerase I is not stimulated by ATP in the presence of the four DNTPs, it is possible that the protein coded by recB gene and/or recD gene is somehow responsible for this stimulation. One possible explanation for ATP-stimulated polymerization is a direct interaction of some recB protein with DNA polymerase I in the presence of ATP. In fact, the behavior observed on addition of

<table>
<thead>
<tr>
<th>Strain</th>
<th>Activity in the absence of ATP/%</th>
<th>Stimulation by ATP/%</th>
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<tbody>
<tr>
<td>C600</td>
<td>100</td>
<td>+65</td>
</tr>
<tr>
<td>1200</td>
<td>130</td>
<td>+61</td>
</tr>
<tr>
<td>W3110 polA1</td>
<td>&lt;5</td>
<td>–</td>
</tr>
<tr>
<td>JC5489 recC22</td>
<td>71</td>
<td>+73</td>
</tr>
<tr>
<td>JC6722 recB21</td>
<td>106</td>
<td>–15</td>
</tr>
</tbody>
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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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acid-insoluble radioactivity/cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP omitted</td>
<td>ATP added</td>
</tr>
<tr>
<td>DEAE-fraction</td>
<td>1020</td>
</tr>
<tr>
<td>DEAE-fraction + <em>pol</em></td>
<td>2613</td>
</tr>
</tbody>
</table>

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 recBC enzyme has been reported previously. 16,21 The polymerase activity of complex isolated from cells of recB21 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


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