The dnaB Protein of Escherichia coli groPB Mutants*

(Received for publication, March 2, 1981)

Eckhard Günther, Erich Lanka, Maria Mikolajczyk, and Heinz Schuster
From the Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, 1 Berlin 33, Ihnesstrasse 63-73, Germany

Escherichia coli groP dnaB mutants are unable to replicate λ wild type phage. They are subdivided into groPA and groPB mutants by their ability to replicate certain λ P mutants, called λA and λB. A functional interaction between dnaB and P protein was deduced from these results. (C. P. Georgopoulos and I. Herskowitz, (1971) in The Bacteriophage Lambda, p. 553, Cold Spring Harbor Laboratory).

In an approach to study the interaction of the replication proteins in vitro, we tried to isolate the dnaB protein of groP mutants by using a φX174 DNA-dependent dnaB complementation assay. However, none of four groPB mutants yielded dnaB activity although dnaB protein could be detected immunologically. Moreover, compared to the groP+ wild type strain, only low amounts of dnaB protein were found in extracts of the groPB mutants B534, B558, and B585. The mutant B612, thermosensitive in the groP character, yielded normal amounts of dnaB protein at 30 °C but low amounts at 40 °C. On the contrary, active dnaB protein could be isolated from all of three groPA mutants in amounts comparable to the groP+ protein.

The dnaB protein of E. coli groPB612 was studied in more detail. It differs from the groP+ protein in vitro in its size, charge, and binding to immobilized ATP. When the groPB phenotype is suppressed by introduction of the plasmid P1bac crr, active dnaB-ban heteromultimers can be isolated, which no longer differ from the groP+ protein with regard to size, charge, and binding to ATP.

The results suggest that the mutation to groPB leads to a labile dnaB multimer in vivo. As a consequence, a lower level of dnaB protein may exist in the cell.

The dnaB protein of Escherichia coli participates both in the initiation and elongation reactions of cellular DNA replication as inferred from studies in vivo and in vitro. (1). Replication of phage λ DNA also depends on the dnaB gene product. An interaction of dnaB with λ P protein has been deduced from both genetic and biochemical studies (2-4). E. coli groPA and groPB mutants were isolated and identified as subclasses of dnaB mutants unable to replicate λ wild type. On the other hand, λ P mutants, called λA and λB, are able to replicate in E. coli groP mutants (2).

Wild type dnaB protein has been purified to homogeneity (5, 6). The protein is a multimer (Mr ~ 290,000) composed of six or seven subunits (5). In an attempt to study the E. coli groP mutants biochemically, we have used a dnaB complementation assay (7-9) for the isolation of the dnaB proteins. The method had been used already for the purification to homogeneity of a mutant dnaB protein (10). Active dnaB protein was isolated from all of three groPB mutants. Surprisingly, the extracts of all groPB mutants tested were inactive in φX174 complementary strand synthesis. Therefore, the groPB dnaB protein was traced immunologically by precipitation with dnaB antibody. Alternatively, the protein was “rescued” in the form of active dnaB-ban heteromultimers isolated from E. coli groPB(P1bac crr) lysogens. The results of these studies are presented in this paper.

MATERIALS AND METHODS

Material, reagents, and methods, unless otherwise indicated, were as previously described (9-11). DEAE-Sepharose (Pharmacia) was used instead of DE52 (Whatman). Buffer A is 20 mM Tris-HCl, pH 7.6, 1 mM MgCl2, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol. Buffer B is 20 mM Tris-HCl, pH 7.6, 1 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.

Bacterial and Phage Strains—The E. coli groP+ strains used were kindly provided by C. P. Georgopoulos (University of Utah, Salt Lake City) and I. Herskowitz (University of Oregon, Eugene) and are listed in Table I. All strains are isogenic with each other (F-, metA-, malB-)

The assays contained [3H]dNTPs, 10 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.

The results of these studies are presented in this paper.

Materials and methods, unless otherwise indicated, were as previously described (9-11). DEAE-Sepharose (Pharmacia) was used instead of DE52 (Whatman). Buffer A is 20 mM Tris-HCl, pH 7.6, 1 mM MgCl2, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol. Buffer B is 20 mM Tris-HCl, pH 7.6, 1 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.

Bacterial and Phage Strains—The E. coli groP+ strains used were kindly provided by C. P. Georgopoulos (University of Utah, Salt Lake City) and I. Herskowitz (University of Oregon, Eugene) and are listed in Table I. All strains are isogenic with each other (F-, metA-, malB-)

The assays contained [3H]dNTPs, 10 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.

Bacterial and Phage Strains—The E. coli groP+ strains used were kindly provided by C. P. Georgopoulos (University of Utah, Salt Lake City) and I. Herskowitz (University of Oregon, Eugene) and are listed in Table I. All strains are isogenic with each other (F-, metA-, malB-)

The assays contained [3H]dNTPs, 10 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.

Bacterial and Phage Strains—The E. coli groP+ strains used were kindly provided by C. P. Georgopoulos (University of Utah, Salt Lake City) and I. Herskowitz (University of Oregon, Eugene) and are listed in Table I. All strains are isogenic with each other (F-, metA-, malB-)

The assays contained [3H]dNTPs, 10 mM MgCl2, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 10% glycerol.
RESULTS

dnaB Protein of E. coli groPB Mutants Is Inactive in In Vitro φX174 DNA Replication

A φX174 DNA-dependent dnaB complementation assay (9) and ATP-agarose affinity chromatography as an essential purification step (10) has been used for the purification of dnaB and PilN protein (17). By this means the dnaB protein of E. coli groP* and the groPA mutants A15, A118, and AT42 also have been purified. Usually purification was started with about 2 units of dnaB activity/mg of Fraction I, equivalent to 2 nmol of dTMP incorporation in 30 min at 30 °C (Table I). Purified dnaB protein migrated as a single band on SDS-gel electrophoresis, as shown on Fig. 1 for groP* and groPA15 indicating a molecular weight of about 60,000.

This value is considerably larger than the 48,000- and 55,000-dalton polypeptide subunits of the dnaB protein reported by others (5, 6). This discrepancy is due to different degrees of cross-linking in the SDS-gel system, as was reported earlier (10).

Extracts of the E. coli groPB mutants B534, B558, and B585 were inactive in φX174 complementary strand synthesis (Table I). Strain groPB612 is a temperature-sensitive groP mutation inhibiting γ growth partially at 37 °C but not at 30 °C (2). This mutant also yielded inactive extracts irrespective of the growth temperature (Table I). No other replication protein seems to be affected since addition of increasing amounts of dnaB wild type protein to Fraction I (120 µg) of all groPB mutants resulted in a corresponding increase in φX174 complementary strand synthesis. The amount of DNA synthesized corresponded to 50 to 110% of that found with Fraction I of BT1071 dnaBts under otherwise identical conditions.

The failure to detect dnaB activity could be due to the absence of dnaB protein in Fraction I. Therefore, dnaB protein was traced by precipitation with dnaB antibody (Fig. 1). About 1 µg of dnaB protein/mg of Fraction I was found for groP* and groPA15, representing a specific activity of about 2000 units/mg of dnaB protein (Table I). A similar value is found for both proteins when purified to homogeneity.

When the groPB mutants B534, B558, and B585 were analyzed, a 5 to 10-fold lower amount of dnaB protein was found (Table I). The failure to detect dnaB activity is, how-

TABLE I

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth temperature °C</th>
<th>dnaB per mg of Fraction I µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>groP*</td>
<td>30</td>
<td>1.9</td>
</tr>
<tr>
<td>groPA15</td>
<td>30</td>
<td>1.4</td>
</tr>
<tr>
<td>groPB534</td>
<td>30</td>
<td>0.1</td>
</tr>
<tr>
<td>groPB558</td>
<td>30</td>
<td>0.2</td>
</tr>
<tr>
<td>groPB585</td>
<td>30</td>
<td>0.1</td>
</tr>
<tr>
<td>groPB612</td>
<td>30</td>
<td>1.0</td>
</tr>
<tr>
<td>groPB612(P1 lac crr)</td>
<td>30</td>
<td>0.4</td>
</tr>
</tbody>
</table>

However, not due to the small amount of dnaB protein. Under the same assay conditions, correspondingly small amounts of dnaB wild type protein in Fraction I stimulate DNA synthesis 10 to 20-fold. The mutant groPB612 is exceptional in this regard in that the amount of dnaB protein found at 30 and 35 °C was comparable to that of groP*. However, only about one-third the amount was found when the strain was grown at 40 °C (Table I). This mutant was chosen for further studies since the quantity of dnaB protein present at 30 to 35 °C made immunological tracing of the protein feasible.

dnaB Protein of groPB612 Differs from groP* in Size, Charge, and Binding to ATP

Ion Exchange Chromatography—dnaB protein can be purified to homogeneity in three steps: ammonium sulfate precipitation, DEAE-Sephalac-, and ATP-agarose chromatography (Fig. 1 and Ref. 10). Therefore, it seemed obvious to use the same procedure for the purification of the dnaB protein from E. coli groPB612. Fraction I of this strain was applied to a DEAE-Sephalac column and the proteins adsorbed were eluted with a linear NaCl gradient. The dnaB protein was traced immunologically, as described under “Materials and Methods.” dnaG- and polC activity was followed on the same column to use their elution profile as marker. A typical analysis is shown on Fig. 2A. The peak fraction of B612 dnaB protein elutes at about 0.17 m NaCl (concomitantly with polC) and with a smear to higher salt concentrations. Under identical conditions, the dnaB protein of groP* is eluted at about 0.34 m NaCl as was also found for the dnaB protein of other E. coli strains (9, 11).

Affinity Chromatography—The difference between groPB612- and the wild type dnaB protein was even more striking when ATP-agarose chromatography was used. When Fraction I (120 mg) from groP* was directly applied to ATPagarose Type 4 (1 ml), more than 95% of the dnaB protein is adsorbed and can be eluted by ATP. On the contrary, 98% of the dnaB protein from B612 (Fraction I) did not bind to ATP-agarose under the same conditions. Similarly, the DEAE-Sephalac fraction (Fig. 2A) was not retained on ATP-agarose. When Type 4 was replaced by ATP-agarose Type 2 or Type 3, more than 88% of the B612 dnaB protein was recovered in the unadsorbed material. Under the same conditions, the

FIG. 1. SDS-gel electrophoresis of dnaB protein. Conditions of electrophoresis were as described previously (9, 21). A to C, Fraction III (ATP-agarose) of dnaB activity from A with BT1071 dnaBts(P1 lac crr) as reference; B, groP* (14 units); C, groPA15 (10 units); D, immunoprecipitate of Fraction I from groPB612 prepared as described under “Materials and Methods.” Protein standards were bovine serum albumin (BSA, M = 68,000), ovalbumin (OVA, M = 43,000), and chymotrypsinogen A (CHYA, M = 25,700).
**DNA B Protein of groPB Mutants Can Be Rescued by P1ban**

Suppression of groPB by P1bac crr—dnaB mutations can be suppressed by either prophage P1bac or P1bac crr (18-20). Suppression of the groPB character by P1bac crr was tested by a comparison of the plating efficiencies of phage λ A and λ A on E. coli groPB612 and its P1bac crr lysogen at different temperatures (Table II). A partial or total suppression was observed at 35 °C depending on whether plating of λ A or λ A was performed.

**Table II**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Suppression of groPB612 by P1bac crr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ A lysozyme (E. coli groPB612)</td>
</tr>
<tr>
<td></td>
<td>λ A lysozyme (E. coli groPB612(P1bac crr))</td>
</tr>
<tr>
<td></td>
<td>Non-lysogenic</td>
</tr>
<tr>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>35</td>
<td>0.8 (+)</td>
</tr>
<tr>
<td>40</td>
<td>0.2 (+)</td>
</tr>
</tbody>
</table>

**Physical Properties—** dnaB protein of E. coli groPB was subjected to glycerol gradient centrifugation. The activity sedimented slightly faster than a catalase marker (M, = 250,000) indicating a sedimentation coefficient of about 11 S (Fig. 3A). The dnaB protein co-sedimented with the activity as revealed by a solid phase immunoassay (Fig. 3B). On the contrary, inactive dnaB protein of groPB612 sedimented somewhat slower than a bovine serum albumin marker (M, = 68,000) with a smear to higher molecular weights (Fig. 3C). Sedimentation of the dnaB peak fraction indicates a sedimentation coefficient of about 5 S corresponding roughly to the molecular weight of the dnaB monomer (M, = ~60,000).

**Figure 2.** DEAE-Sepharose column chromatography of groPB and dnaB and groPB dnaB-P1ban protein. A: Fraction I (616 mg of protein) of E. coli groPB612, grown at 30 °C, was diluted into Buffer B to yield 5 mg of protein/ml. The solution was applied to a column (2.6 x 11.3 cm) of DEAE-Sepharose equilibrated in Buffer B (ATP omitted). Protein was eluted with a linear NaCl gradient (900 ml, 0.05 to 0.6 M NaCl in Buffer B, 22 ml/fraction). The dnaG and polC activity was measured as described under “Materials and Methods.” The position of dnaB* protein was determined from the elution profile of the dnaB complementing activity of E. coli GroP+ subjected to the same procedure.

A set of subsequent fractions were pooled, ammonium sulfate (0.369 g/ml) was added, and the precipitate collected by centrifugation after 1 h of stirring and overnight incubation at 0 °C. The pellet was dissolved in Buffer B and dialyzed against the same buffer. An aliquot of each subfraction was then treated with dnaB antiserum.

**Figure 3.** Glycerol gradient centrifugation of dnaB protein from E. coli groPB and groPB612. Glycerol gradients (3.7 ml, 18 to 43%, w/v) containing 20 mM Tris-HCI (pH 7.6), 50 mM NaCl, 10 mM MgCl₂, 0.1 mM EDTA, 2 mM dithiothreitol, 2 mM ATP (Tris-salt), and 0.1% Brij 58 were prepared in polyallomer tubes: (i) Fraction III (30 µl, 3 units of dnaB) of groPB, (ii) DEAE-Fraction (30 µl), 49 µg of protein; (iii) the reference proteins catalase (CAT), alcohol dehydrogenase from yeast (ADH), and bovine serum albumin (BSA) were layered onto gradients which were run in parallel. Centrifugation was performed in a Beckman SW 60 Ti rotor for 16 h at 2 °C at 47,000 rpm. Fractions (0.27 ml) were collected from the tube bottom. A, the dnaB complementing activity was measured as described (9). The position of the reference proteins was determined by SDS-gel electrophoresis. A solid phase immunoassay (16) was used to visualize dnaB protein. An aliquot of each fraction (B, groPB, 10 µl; C, groPB612, 3 µl) was subjected to SDS electrophoresis and the proteins were transferred electrophoretically to a sheet of nitrocellulose membrane. The immobilized proteins were reacted for 2 h with a diluted (1:250) sheep anti-dnaB serum (11). The membrane was washed, incubated for 30 min in a diluted (1:50) fluorescein-conjugated rabbit anti-sheep IgG serum (Byk-Mallinkrodt), and washed again. Photographs of the membrane were taken under UV-light (253 nm) through a yellow filter using a Polaroid camera and film type 665.
\( \lambda^- \) is taken as basis. No suppression or only a partial suppression was observed at 40 °C using the same \( \lambda \) phages. On the contrary, the plating efficiency of X' was the same irrespective of the strain and the temperature (Table II). This reduction or loss of suppressibility with increasing temperature is parallel to a corresponding reduction of the total amount of dnaB + ban protein recovered from the groPB612(P1bac crr) lysogen (Table I).

**Heteromultimers of groPB dnaB-ban—** Fraction I of E. coli groPB612(P1bac crr) grown at 30 °C was subjected to DEAE-Sephasel chromatography (Fig. 2B). The main peak of dnaB and ban protein eluted at about 0.29 M NaCl exhibiting only a very low activity. A minor peak of inactive dnaB protein is eluted at about 0.17 M NaCl, as was shown before for the nonlysogenic strain (Fig. 2A). The main peak of dnaB-ban activity comes off the column at about 0.39 M NaCl. This is the fraction containing the largest amount of ban relative to dnaB protein (Fig. 2B). When the groPB612(P1bac crr) lysogen was grown at 35 °C and subjected to the same procedure, a similar elution pattern was obtained. However, the main peak of the dnaB-ban activity contained about twice as much ban as dnaB protein (data not shown).

Active fractions eluting at about 0.39 M NaCl from the DEAE-Sephasel column were pooled (Fraction II) and applied to ATP-agarose. The dnaB-ban activity and protein were determined in the unabsorbed material as well as in the ATP eluate. The results obtained for E. coli groPB612(P1bac crr) grown at 30 and 35 °C are shown on Fig. 4. Common to both analyses is the following: dnaB and ban protein are found in the unadsorbed material as well as in the ATP eluate.

![Figure 4](https://example.com/figure4.png)

**Discussion**

A subdivision of E. coli groP mutants into groPA and groPB derived from genetic data (2) gains some support from the biochemical analysis presented in this paper. Whereas active dnaB protein could be isolated from all of three groPA mutants in amounts comparable to that obtained from groP', all of four groPB mutants, on the contrary, yielded dnaB protein which was inactive in \( \delta \times 174 \) complementary strand synthesis. groPB dnaB protein also did not support the in vitro replication of double-stranded DNA. Addition of ColEl DNA to an extract from groP' stimulated DNA synthesis, whereas an extract from groPB612, grown at 30 °C, was inactive (data not shown).

Moreover, groPB mutants yield low amounts of dnaB protein (Table I). A striking example is the mutant groPB612. In accordance with its groPB character being temperature-sensitive (2), normal and low amounts of dnaB protein are found at 30 to 35 and 40 °C, respectively (Table I). It is not
yet known whether these findings reflect the \textit{in vivo} conditions. The existence of \textit{groPB612 dnaB} protein as a monomer \textit{in vitro} (Fig. 3) suggests that \textit{groPB} \textit{dnaB} multimer molecules may also be unstable \textit{in vivo} and, therefore, might be subjected to degradation. As a consequence, the number of \textit{dnaB} molecules in a cell might become limited and replication of \textit{X} might be impaired without affecting cellular DNA replication. If only the relative levels of \textit{dnaB} and \textit{P} protein are crucial for a productive \textit{X} replication, then the mutation to \textit{XaB} must result in an adjusted level of \textit{P} protein which is different from that of \textit{X} wild type or \textit{XaA} mutants.

Suppression of \textit{E. coli dnaB} mutants by prophage Pl1bac or Pl1bac \textit{crr} was explained by the interaction of \textit{dnaB} and \textit{ban} protein subunits in the form of heteromultimers (9, 11). This model is strongly supported by the results of ATP-agarose chromatography of \textit{dnaB} and \textit{ban} protein from \textit{E. coli groPB612} and its \textit{Pl1bac crr} lysogen. \textit{groPB dnaB} protein, which \textit{per se} does not bind to ATP-agarose, is bound via \textit{ban} protein, provided that the latter is present in at least twice the molar amount of \textit{dnaB} protein (Fig. 4). These results suggest that Fraction II of \textit{E. coli groPB612(Pl1bac crr)} contains a population of \textit{dnaB-ban} heteromultimers which is partitioned by affinity chromatography. Heteromultimers containing more \textit{ban} than \textit{dnaB} subunits are bound to ATP, whereas those containing more \textit{dnaB} than \textit{ban} are unable to bind. The instability of \textit{groPB612 dnaB-ban} heteromultimers (Table III) contrasts with the noticeable stability of heteromultimers composed of \textit{ban-} and \textit{dnaB-} or \textit{dnaBts} subunits (9, 11, 17). Apparently the interaction of \textit{ban-} and \textit{groPB612 dnaB} subunits is also impaired, as it is assumed for the \textit{groPB dnaB} subunits among one another. This may also explain the low recovery of \textit{dnaB} protein from \textit{groPB612(Pl1bac crr)} at 40 °C in spite of the presence of \textit{ban} protein in the cell (Table I).

Acknowledgments—We are much indebted to C. P. Georgopoulos, I. Herskowitz, R. D’Ari, and D. Touati-Schwartz for supplying us with \textit{E. coli groP} strains, phage \textit{lambda} and \textit{P1} mutants. We thank W. Staudenbauer for the advice in using the ColEl DNA replication system. We are grateful to M. Meuthen for expert technical assistance.

REFERENCES