The molecular mechanism by which *Escherichia coli* dnaB mutations are suppressed by prophage Plbac has been investigated. The prophage P1 codes for a dnaB analog (ban) protein which is repressed in cells containing P1 wild type and expressed constitutively in cells containing the mutant Plbac. Plbac ban mutants fail to synthesize an active ban protein (D’Ari, R., Jaffé-Brachet, A., Touati-Schwartz, D., and Yarmolinsky, M. B. (1975). J. Mol. Biol. 94, 341-368; Ogawa, T. (1975) J. Mol. Biol. 94, 327-340). An enzyme has been isolated from Plbac lysogenic *E. coli* strains which complements an extract of a thermosensitive (ts) *E. coli* dnaB mutant assayed for *in vitro* DNA synthesis. Lysogens of a dnaB amber mutant are used as enzyme source in which the amber mutation is either un-suppressed (su-) or suppressed by the suppressors supE or supF resulting in a thermosensitive and thermostable dnaB protein, respectively (Ogawa, T. (1975) J. Mol. Biol. 94, 327-340).

Highly purified enzyme preparations from all strains tested revealed native molecular weights for the enzyme of approximately 260,000 as estimated by glycerol gradient centrifugation. The enzyme isolated from Plbac lysogens of the *E. coli* supE and supF strains is composed of polypeptides with molecular weights of 62,000 + 56,000 and 61,000 + 56,000, respectively. The larger components (M₀ = 62,000 and Mₐ = 61,000) are presumably the dnaB monomers synthesized by supE and supF strains. The 56,000 dalton polypeptide is absent in nonlysogens, P1 wild type, and Plbac ban lysogens, and it is the only component of the 260,000 dalton enzyme found in the purified preparation from a dnaB su-(Plbac) lysogen. It has therefore been tentatively identified as the P1 ban protein.

In Plbac lysogens dnaB and ban monomers are assumed to be associated with one another in heteromultimers. The enzymatic activity of such heteromultimers isolated from the supE(Plbac) lysogens is thermolabile in contrast to the thermostable heteromultimers from supF(Plbac) lysogens. It appears that suppression of the supE dnaB ts mutation by Plbac results from a stabilization of the dnaB ts polypeptide by ban subunits in a heteromultimer. The heteromultimers contain a DNA-dependent and -independent ribonucleoside triphosphatase activity. Both the dnaB complementing and the DNA-dependent ribonucleoside triphosphatase activity are inactivated by antibodies directed against dnaB.

During P1 infection of an *Escherichia coli* dnaB temperature-sensitive mutant at the nonpermissive temperature synthesis of ban protein enables the phage to grow (3), and bacterial DNA synthesis recovers transiently (4). Synthesis of ban protein is repressed in the wild type P1 prophage and expressed constitutively in Plbac mutants. Plbac lysogens of *E. coli* dnaB ts mutants are, therefore, able to grow at temperatures nonpermissive for the nonlysogens (1, 2). Plbac ban mutants code for a defective ban protein and therefore fail to replicate in *E. coli* dnaB ts mutants at elevated temperatures. As prophage they do not support growth of dnaB ts hosts at the nonpermissive temperature (1).

In an attempt to experimentally investigate the suppression of *E. coli* dnaB ts mutants by prophage Plbac, the phenomenon was simulated in an *in vitro* system for DNA replication employing the conversion of φX174 single-stranded DNA to its duplex form (5, 6). Duplex DNA synthesis is dependent, among other proteins, on dnaB protein (7, 8). With crude enzyme fractions from Plbac lysogens we observed that Plbac protein permitted temperature-resistant φX174 DNA duplex DNA synthesis when the reaction mixture contained a temperature-sensitive dnaB protein (5).

The present report describes the purification of the P1ban protein using as an assay the stimulation of φX174 DNA-dependent DNA synthesis in inactivated extracts of *E. coli* dnaB ts cells (7, 8). The *E. coli* dnaB protein itself has been purified by means of this method (8, 9). As a source for ban protein, Plbac lysogens of the *E. coli* dnaB266 mutant and its derivatives were chosen for the following reason. Strain 266, originally isolated as a dna ts mutant (10) was found to be a dnaB amber mutation that is lethal to the host when not suppressed and only conditionally lethal (ts) when suppressed by supE (2). Suppression of dnaB266 by supF results, however, in the synthesis of a temperature-resistant (tr) dnaB protein (2). In addition, the P1bac prophage permitted construction of an otherwise inviable strain bearing the unsuppressed 266 amber mutation (1). Thus, these strains harbor P1ban protein in combination with a dnaB ts, a dnaB tr, or a dnaB amber protein in an otherwise nearly isogenic background facilitating comparative studies.

**MATERIALS AND METHODS**

**Phage and Bacterial Strains**

Phage P1Cm1, P1Cm1bac-1, and P1Cm1bac-I ban-1 (1) were provided by A. Jaffé-Brachet and D. Touati-Schwartz (Institut de Recherche en Biologie Moléculaire Université Paris VII-Tour 43, 2 place Fussieu, F-75221 Paris, Cedex 05, France) and are designated P1, P1bac, and P1bac ban. P1 Tc1 (11) was provided by W. Arber (Bioszentrum der Universität Basel, Abt. Mikrobiologie, Klingenbergstr. 70, CH-4056 Basel, Switzerland). The chloramphenicol (tetacycline) resistance of P1Cm1 (P1 Tc1) serves as a convenient

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*The abbreviations used are: ts (tr), temperature sensitive (resistant); am, amber; SDS, sodium dodecyl sulfate.*

**Revised**

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selective indicator of prophage P1 (1, 11). T4 D', T4 D amB17 (suppressible by supD and supE, not by supF), T4 D amM619 (suppressible by supD and supE, not by supE), and T4 D am#41 (suppressible by supD, supE, and supF) were provided by D. Touati-Schwartz (1). The E. coli K12 strains of the Q series (1) were provided by D. Touati-Schwartz and are listed in Table I. All strains carry the dnaB266 amber mutation either in its unsuppressed (su·) or suppressed (supF or supE) form in an otherwise isogenic background (F', thy', thi, tyr, lac, lacI). The strain with the unsuppressed (su·) mutation is only viable as Plbac lysogen (1, 2). Pl wild type and Plbac ban lysogens of strain Q1710 and Q1650 were isolated in strains including Q1650 (Table I). Strains were tested for the presence or absence of suppressors by the ability to suppress the T4 amber mutations mentioned above. The dnaB protein is phenotype of strain Q1650 was routinely checked by superinfection with P1 Tc1 and selection at 30°C of tetracycline-resistant colonies as described (11). The latter were then tested for temperature-sensitive growth. Strain BT1071 dnaB was used for the preparation of extracts for dnaB complementation assays (7, 8).

Growth of Cells and Preparation of Extracts

Bacteria were grown in TY medium (5) at a constant pH (7.5) in a 100-liter Bioengineering Fermentor to about 3 x 10^10 cells/ml and harvested as described (5). The growth temperature was 25°C for temperature-sensitive strains and 40°C for temperature-resistant strains; all strains were grown in TY medium supplemented with chloramphenicol (10 µg/ml). Bacteria were lysed according to the lysosome-spermintine procedure (12) with modifications as described under "Results."

Assays

DNA synthesis with ammonium sulfate fractions was measured as described (5).

dnaB Complementation—The E. coli BT1071 dnaB ammonium sulfate fraction was prepared as described for strain BC1304 (13). Fractions (20 to 30 mg of protein/ml) were heated for 5 min at 37°C to inactivate residual dnaB ts protein. Eighty to 140 µg of protein was used per assay. DNA synthesis was followed for 30 min at 37°C as described (5) with 370 µmol (nucleotides) of αX174 DNA. One unit of dnaB complementing activity incorporates 1 nmol of 32P, under the above conditions.

Assay for DNA-dependent ATPase—Conditions were as described (14) except that 1.5 nmol (nucleotides) of αX174 DNA was used and the reaction terminated after 30 min at 25°C. One unit of ATPase catalyzed the production of 1 µmol of P_i under the above conditions.

Chemicals

Agarose-A11', Type 4 was obtained from Bio-Rad Laboratories (Milwaukee); [y-^32P]ATP, 2 Ci/mmol, was from The Radiochemical Centre, Amersham. Tricine base and catalase, 39,000 units/mg (w/v) were obtained from Worthington, Freehold, N.J. Other chemicals were as described previously (5, 13).

Buffers

Buffer A consisted of 20 mM Tris·HCl (pH 7.6), 50 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM ATP, 12% (w/v) glycerol; Buffers B to E were Buffer A with the following modifications (in parentheses): B (1 mM MgCl2, C (MgCl2, and ATP omitted), D (5 mM MgCl2 and 5 mM ATP), and E (1 mM ATP, glycerol omitted).

RESULTS

DNA Synthesis with Ammonium Sulfate Fractions of Strains Derived from E. coli dnaB266

Ammonium sulfate fractions were prepared from P1 lysogenic and nonlysogenic strains as described in the following section (Fraction I). They were tested for their ability to catalyze the conversion of αX174 DNA to its duplex form at different temperatures. The rationale of these experiments was to simulate, in vitro, the in vivo suppressing action of P1ban protein as described earlier (5) and to determine conditions which would distinguish between P1ban and E. coli dnaB-mediated αX174 duplex DNA synthesis. Use of these conditions would subsequently permit the assay of P1ban protein specific activity during further purification.

Maximum DNA synthesis at 25°C usually was obtained with between 90 and 150 µg of protein of Fraction I. The amount of protein depended on the mutant strain used as enzyme source and the DNA-synthesizing capacity of the ammonium sulfate fraction. Following a short lag period, DNA synthesis was linear for about 30 min at 25°C (data not shown). The temperature sensitivity was determined by following DNA synthesis for 20 min at temperatures ranging from 25 to 42°C with submaximal protein concentrations (60 to 120 µg per assay). The relative amounts of DNA synthesized at different temperatures (25°C = 1.0) are shown in Fig. 1. The extent of DNA synthesis at higher temperatures differs appreciably between fractions containing a temperature-resistant dnaB protein (E. coli supF and supF(P1bac)) and those containing a temperature-sensitive dnaB protein not suppressed by P1bac (E. coli supE and supE(P1bac ban)). DNA synthesis with a fraction from E. coli supE(P1bac) was more temperature-resistant than with a fraction containing a temperature-sensitive dnaB protein but less resistant than with a fraction containing a temperature-resistant dnaB protein (Fig. 1).

The different temperature resistance of dnaB complementing activities isolated from the P1bac lysogens of E. coli supF and of supE (Fig. 1) persisted upon further purification as shown below (Fig. 4). However, among the fractions from the E. coli supE series the relatively greater heat stability of the P1bac lysogen disappeared on further purification (data not shown). Moreover, in this series of strains the yields of dnaD units were low as shown below (Table III). These findings prevented the use of this assay with supE strains to measure P1ban protein specific activity during its purification. We, therefore, attempted to purify and characterize the dnaB complementing activity from strains of the E. coli supF series. These strains contain a temperature-resistant dnaB protein and, in comparison to the E. coli supE strain, the yields of...
Ammonium Sulfate Fractionation—Finely ground ammonium sulfate (0.226 g/ml) was slowly added to the crude extract with stirring. Stirring was continued for 30 min, and the precipitate was then collected by centrifugation at 10,000 rpm for 20 min in a Sorvall GSA rotor. The pellet was washed with 50 ml of ammonium sulfate (0.298 g/ml), dissolved in Buffer A, and dialyzed for 3 h against two 1-liter changes of Buffer A (Fraction I, 26.5 ml).

**DEAE-cellulose**—Fraction I was diluted with Buffer A to 200 ml and applied to a column (3 x 35 cm) of DEAE-cellulose equilibrated with Buffer A (ATP omitted). The dnaB complementing activity was eluted between 0.28 and 0.38 M NaCl with a linear NaCl gradient (3000 ml, 0.05 to 0.6 M NaCl in Buffer A, 29 ml per fraction). Fractions containing dnaB complementing activity were pooled, ammonium sulfate (0.369 g/ml) was added, and the precipitate collected by centrifugation as described above. The pellet was dissolved in 3 ml of Buffer B and dialyzed 3 h against two 250-ml changes of Buffer B (Fraction II, 3.6 ml).

**Glycerol Gradient Sedimentation**—Fraction II (1.6 ml) was diluted with Buffer C to 8 ml and applied to a column (0.5 x 5 cm) of agarose-ATP equilibrated with Buffer C. The column was washed with Buffer C (5-fold bed volume), and the dnaB complementing activity was eluted with Buffer D and collected in 0.5-ml fractions (Fraction III, 2.5 ml).

**Glycerol Gradient Sedimentation**—Fraction III (0.2 ml) was layered on a 4.8-ml glycerol gradient and centrifuged as described under "Materials and Methods" (Fraction IV, 1.6 ml).

**Physical Properties of dnaB Complementing Activity**—Glycerol gradient centrifugation of the dnaB complementing activities from *E. coli* supF, supF(P1bac), and supF(P1bac ban) gave identical sedimentation coefficients of about 12 S. An estimation of the native molecular weight was calculated according to the equation 

$$\text{MW}_n = \left( \frac{S^n}{S_0^n} \right) \left( \frac{\text{MW}}{\text{MW}_n} \right)^{1/3}$$

(19, 20) with β-galactosidase (molecular weight 515,000) and catalase (molecular weight 250,000) as standards (Fig. 3 and data not shown). Values of about 340,000 (β-galactosidase as standard) and 260,000 (catalase as standard), respectively, were obtained by this method. The latter value agrees with the native molecular weight of the dnaB protein reported by others (8, 9).

Fraction III of *E. coli* supF(P1bac) when subjected to SDS gel electrophoresis yielded four major polypeptides with estimated molecular weights of 70,000, 61,000, 66,000, and 38,000, respectively (Fig. 2). Only the 61,000 and 56,000 dalton polypeptides (peak Fraction III) (Fig. 3) were observed. The weight ratio of the 61,000 and the 56,000 dalton polypeptides (peak Fraction III) (Fig. 3) was used to calculate the molecular weight of the dnaB protein.

**Table II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Units</th>
<th>Total Protein</th>
<th>Specific Activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Ammonium Sulfate</td>
<td>2,870</td>
<td>756</td>
<td>3.8</td>
<td>100</td>
</tr>
<tr>
<td>II. DEAE-cellulose</td>
<td>905</td>
<td>6.6</td>
<td>137</td>
<td>32</td>
</tr>
<tr>
<td>III. Agarose-ATP*</td>
<td>1,180</td>
<td>0.45</td>
<td>2,820</td>
<td>41</td>
</tr>
<tr>
<td>IV. Glycerol gradient*</td>
<td>685</td>
<td>0.115</td>
<td>5,870</td>
<td>24</td>
</tr>
</tbody>
</table>

*These steps were performed with only a part of the fraction from the preceding purification step. The values reported assume that the yield and purification would be the same if the entire DEAE-cellulose and agarose-ATP fractions were subjected to the following procedure(s).
The *dnaB* complementing activity from *E. coli* supF was purified about 500-fold in the same manner as described above for strain supF (P1bac). Starting with 269 g of wet cell paste Fraction IV contained a total of 900 units of *dnaB* (specific activity, 2,040 units/mg). The yield was 11%. Fraction III of this strain when subjected to SDS-gel electrophoresis demonstrated the presence of three major proteins with molecular weights 70,000, 61,000, and 38,000, respectively. A polypeptide of molecular weight 56,000 was not found (Fig. 2). Upon glycerol gradient centrifugation only the 61,000 dalton polypeptide co-sedimented with the *dnaB* complementing activity (Fig. 3).
The dnaB complementing activity from E. coli supF(P1) was purified as described above. Starting with 315 g of wet cell paste, Fraction III contained a total of 350 units of dnaB (specific activity, 1,600 units/mg). The yield was 8%. SDS-gel electrophoresis again demonstrated the presence of three major polypeptides having the same molecular weights as the corresponding polypeptides isolated from E. coli supF. A polypeptide of molecular weight 56,000 was not detected in extracts from the P1 lysogen (see Fig. 5). Upon glycerol gradient centrifugation, again only the 61,000-dalton polypeptide co-sedimented with the dnaB complementing activity (data not shown). From these results it is concluded that the 61,000-dalton polypeptide is a subunit of the E. coli dnaB protein. The 56,000-dalton polypeptide synthesized by E. coli supF(P1bac) is tentatively identified as a subunit of the P1ban protein.

Purification of dnaB Complementing Activity from Strains of E. coli supE dnaB ts Series

The dnaB complementing activities from E. coli supE, supE(P1bac), and supE(P1bac ban) were purified as described above. The results are summarized in Table III. The specific activities of corresponding fractions isolated from the different strains of the E. coli supE series are similar (Table III). However, in most cases the values are significantly lower when compared with the corresponding activities obtained from strains of the E. coli supF series (see above, and Table II). In addition, the total units of dnaB obtained were always low irrespective of whether or not the dnaB ts mutation is suppressed by the prophage P1bac (Table III). This suggests that even under the conditions of effective suppression in vitro the resulting in vitro dnaB complementing activity may be due to the presence of dnaB ts subunits which are somewhat unstable. The fact that the in vitro DNA synthesis with Fraction I from E. coli supE(P1bac) was more temperature sensitive than that with E. coli supF(P1bac) (Fig. 1) supports this proposition. Further support for this suggestion was obtained when the heat stability of the dnaB complementing activities from E. coli supE(P1bac) and from E. coli supE(P1bac ban).

### Table III

| Purification of dnaB complementing activity from E. coli supE and its P1 lysogens |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Fraction        | Total units     | Total protein   | Specific activity | Yield           |
|                 | mg             | units/mg        |                  | %              |
| E. coli supE    | 800            | 388             | 2.3              | 100             |
| I. Ammonium sulfate | 800           | 388             | 2.3              | 100             |
| II. DEAE-cellulose | 225           | 18.5            | 12               | 25              |
| III. Agarose-ATP  | 275            | 0.43            | 640              | 31              |
| IV. Glycerol gradient | 30          | 0.012           | 2,500            | 3.4             |

| E. coli supE (P1bac) |
|-----------------|-----------------|-----------------|-----------------|
| I. Ammonium sulfate | 542            | 1,530           | 0.35            | 100             |
| II. DEAE-cellulose | 76             | 6.9             | 11               | 14              |
| III. Agarose-ATP  | 62             | 0.09            | 689              | 11              |
| IV. Glycerol gradient | 18           | 0.008           | 2,250            | 3               |

| E. coli supE (P1bac ban) |
|-----------------|-----------------|-----------------|-----------------|
| I. Ammonium sulfate | 542            | 264             | 1.9             | 100             |
| II. DEAE-cellulose | 36             | 0.3             | 5.7             | 7.2             |
| III. Agarose-ATP  | 37             | 0.05            | 740              | 7.4             |
| IV. Glycerol gradient | 24           | 0.01            | 2,400            | 4.8             |

When the dnaB complementing activity was extracted from E. coli su (P1bac) the specific activity of Fraction I was always much lower than the corresponding fraction isolated

![Fig. 4: Heat inactivation of dnaB complementing activity.](http://www.jbc.org/content/240/1/4750/F1.large.jpg)
from all other strains tested. Upon further purification the activity was lost even more rapidly than with extracts from E. coli supE strains (Table IV). SDS gel electrophoresis demonstrated the presence of large amounts of polypeptides of molecular weights 70,000 and 38,000. A polypeptide of molecular weight 56,000 was present in trace amounts. No polypeptide was detectable at the position at which the dnaB protein was to be expected (Fig. 5). The dnaB complementing activity sedimented by glycerol gradient centrifugation with a velocity corresponding to a molecular weight of about 260,000 (catalase as standard). A co-sedimentation of the 56,000-dalton polypeptide with the dnaB complementing activity could not be demonstrated unequivocally due to the low levels of this polypeptide in Fraction III. The size of the dnaB amber peptide synthesized in the su′(P1bac) strain is unknown. The absence of a polypeptide in the molecular weight range of 61,000 to 62,000 in the nonsuppressed dnaB strain strongly supports the assumption that the polypeptides of this size class in the E. coli supE and supF suppressor strains are the dnaB protein molecules (Fig. 5).

**TABLE IV**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total units</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Ammonium sulfate</td>
<td>55</td>
<td>410</td>
<td>0.13</td>
<td>100</td>
</tr>
<tr>
<td>II. DEAE-cellulose</td>
<td>31</td>
<td>3.7</td>
<td>8</td>
<td>56</td>
</tr>
<tr>
<td>III. Agarose-ATP</td>
<td>1.3</td>
<td>0.02</td>
<td>65</td>
<td>2.4</td>
</tr>
<tr>
<td>IV. Glycerol gradient</td>
<td>0.3</td>
<td>nm*</td>
<td>nm*</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*nm, not measurable.

**FIG. 5.** SDS-gel electrophoresis of dnaB complementing activity of strains derived from E. coli supE(Plbac) strain is unrecognizable. The number in parentheses indicates ATPase activity without φX174 DNA.

**TABLE V**

<table>
<thead>
<tr>
<th>Fraction IV</th>
<th>ATPase activity</th>
<th>dnaB Complementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli supF</td>
<td>+ 0.79</td>
<td>19.7</td>
</tr>
<tr>
<td>E. coli supF(P1bac)</td>
<td>+ 3.63</td>
<td>66.5</td>
</tr>
</tbody>
</table>

* The assay contained 12 mM N-ethylmaleimide.

**TABLE VI**

<table>
<thead>
<tr>
<th>Fraction III</th>
<th>dnaB Activity</th>
<th>ATPase</th>
<th>dnaB Complementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli supF</td>
<td>- 100 (14.4)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E. coli supF(P1bac)</td>
<td>+ 18.8 (15.2)</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

An ATPase activity co-purifies with the dnaB complementing activity from E. coli supF and supF(P1bac) (Fig. 5). The ATPase and the dnaB complementing activity (Fraction IV) from both strains are resistant to N-ethylmaleimide (Table V and data not shown). The ATPase activity from the P1bac lysogen differs from the ATPase activity of the nonlysogenic strains of E. coli supF and supF(Plbac) in that the DNA-independent enzyme activity is relatively higher in the lysogen than in the nonlysogen (Table V). The same characteristic difference was found between the ATPases of the P1bac and the corresponding nonlysogenic strains of E. coli supE (Ref. 21, and data not shown) and of E. coli supF(Plbac) (22). The strong DNA-independent ATPase activity seems to depend on the presence of the Plbac protein as it is not found in the P1 wild type lysogen of E. coli supF (Fraction IV) and in the P1bac lysogen of E. coli supE (Fraction IV). The ATPases of the latter two strains are strongly stimulated by φX174 DNA as was found with the enzymes of the corresponding nonlysogens. Antibody to dnaB inactivates both the dnaB complementing activities from E. coli supF and supF(P1bac) and the φX174 DNA-dependent ATPase activity. Antibody to dnaB does not inactivate the DNA-independent ATPase activity (Table VI). These results clearly demonstrate that the DNA-dependent ATPase is associated with the dnaB and dnaB Plbac protein, respectively.

**DISCUSSION**

Two proteins composed of polypeptides of slightly different peptide molecular weights (56,000 + 62,000 and 56,000 +
61,000) co-purify with the dnaB complementing activity from P1ban lysogens of E. coli supE and supF. The smaller subunit (M₀ = 56,000) is tentatively identified as the P1ban protein for the following reasons: 1) it is absent in the corresponding nonlysogenic strains; 2) it is synthesized under the control of P1bac as it is not found in a P1 wild type lysogen in which its synthesis is repressed (1); 3) it is not found in a P1bac ban lysogen in which ban protein or its synthesis is defective (1); 4) it is presumably the only protein which contributes to the dnaB complementing activity from an E. coli su-(P1bac) lysogen. The larger subunit (M₀ = 61,000 from E. coli supF, M₀ = 62,000 from E. coli supE) most likely is the dnaB polypeptide. A polypeptide of this molecular weight is absent from the dnaB complementing activity from E. coli su-(P1bac). The slight but distinct difference in the molecular weight of the dnaB polypeptide molecules of E. coli supE and of supF is not understood since the polypeptides should differ by only the amino acids glutamine and tyrosine. The dnaB complementing activity of another strain, E. coli dnaB252(P1bac), also yielded two polypeptides with molecular weights of 60,000 (dnaB252) and 56,000 (P1ban) (22). For molecular weight determinations care was taken to analyze different dnaB preparations on the same gel. It is noteworthy that even with these precautions dnaB protein molecules from three different E. coli strains reproducibly revealed slightly different peptide molecular weights (Fig. 5, and Ref. 21). The dnaB polypeptide of a fourth strain, E. coli BT1000, was found to have a molecular weight of 60,000. As expected, in all corresponding P1bac lysogens the molecular weight (56,000) of the P1ban polypeptide was identical. The nature of the 70,000 and 38,000 dalton polypeptides appearing in variable amounts in Fractions III (Fig. 5) is unknown. These polypeptides do not have an ATPase activity. When bound to agarose-ATP they can be eluted by AMP ahead of the dnaB protein as was found previously (unpublished results).

The dnaB and ban subunits are apparently associated with one another in a heteromultimer with a native molecular weight of at least 260,000 as shown by glycerol gradient centrifugation. The fact that antibodies directed against dnaB inactivate the dnaB complementing activity and the υX174 DNA-dependent ATPase of dnaB-ban heteromultimers and of dnaB homomultimers to the same extent supports this observation. The dnaB complementing activity from E. coli supF(P1bac) is thermolabile when compared with that of E. coli supF(P1bac). This could result from an interaction of the dnaB ts- and P1ban subunits leading to an inactivation of the associated P1ban subunits. The specific activities of dnaB ts- and dnaB252 to P1ban complexes from E. coli supF are similar and low when compared with those of supF strains. Evidently, the presence of dnaB ts subunits impairs the activity of the whole molecule irrespective of whether or not the dnaB mutation is suppressed by P1bac (Table III).

When the P1bac lysogens of E. coli supF and supE are grown at 40°C, i.e. under conditions of effective suppression of the dnaB ts mutation by the prophage P1bac, the relative numbers of ban:dnaB subunits in the heteromultimers isolated are 2:5 and 2:3, respectively. A ratio of 2:3 was also found in heteromultimers from E. coli dnaB252(P1bac) grown at 40°C (22). If these numbers reflect the overall composition of the heteromultimers in vivo, it implies that one ban subunit can stabilize more than one dnaB subunit in a heteromultimer. Suppression of the dnaB ts mutation by P1bac may be based on such a stabilization. It is not known whether ban protein free of dnaB is as effective as dnaB protein alone in initiating υX174 DNA complementary (−) strand synthesis (23), and the failure to isolate dnaB complementing activity from E. coli su-(P1bac) in amounts comparable to that from su− strains may point to the ineffectiveness of the ban protein in the total absence of dnaB protein. It may therefore be that the assay used in our purification procedure selects against multimers predominantly or solely composed of ban subunits although such multimers may be the most effective molecules in vivo.

An ATPase activity co-purifies with the dnaB complementing activity of all strains so far tested. The presence of the ban protein has a pronounced effect in that the DNA-independent constituent of the total ATPase activity is higher. The same effect was observed between the ATPases of the dnaB complementing activities from E. coli dnaB252 and its P1bac lysogen (22). This property may be due to either an alteration of the dnaB ATPase by the ban protein or an association of an ATPase activity with P1ban protein which is different from that of dnaB protein. It is interesting to note that the DNA-independent ATPase activity of both the dnaB protein and the dnaB ban complex is not inactivated by antibody to dnaB. The antibody molecules may not cover the active site of the enzyme sufficiently to prevent hydrolysis of ATP molecules although preventing the enzyme from interacting with DNA.

Our results for the molecular weight of oligomeric form of dnaB protein agree fairly well with previously reported results, but the subunit molecular weight (61,000 to 62,000) is appreciably different from that reported by others (6, 9, 24). The specific activities of homogeneous dnaB or dnaB ban preparations containing a temperature-resistant dnaB protein (Table II, and data not shown) are comparable to those reported for the wild type dnaB protein of E. coli HMS83 (8, 9, 24). An ATPase activity that is stimulated 10-fold by DNA was found to be associated with the dnaB protein of strain HMS83 (114). From an analysis of E. coli dnaB heteroallelic diploids the existence of a functional multimeric aggregate of dnaB gene products that is at least a tetramer was deduced (25). In a P1bac lysogen ban subunits apparently associate with the dnaB subunits at random as it is assumed for dnaB subunits in a nonlysogen (25).

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