Stabilization by ATP and ADP of *Escherichia coli* dnaB Protein Activity*

Eckhard Günther, Maria Mikolajczyk, and Heinz Schuster

From the Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, 1 Berlin 33, Germany

(Received for publication, August 17, 1981)

The effect of adenine ribonucleotides on the stability of *Escherichia coli* dnaB protein in cellular crude extracts was studied. Stabilization of dnaB protein by ATP or ADP, but not by AMP, was manifested in that (i) the activity and yield of wild type dnaB protein is enhanced in the presence of ATP, (ii) the dnaB protein of *E. coli* dnaB mutants, such as groPB and dnaB352/ColEI1: dnaAc+, which is inactive in a dnaB complementation assay, can be isolated in active form in the presence of ATP or ADP, (iii) ATP or ADP protect the dnaB protein of an *E. coli* dnaBts mutant from inactivation at 37 °C, and (iv) inactive groPB and dnaBts protein can be reactivated partially by ATP. Thus, the stabilizing effect of ATP and ADP can be exploited for the isolation of otherwise inactive or labile mutant dnaB proteins.

The dnaB protein of *Escherichia coli* is essential throughout the replication cycle of the bacterial chromosome (1). It interacts in vitro with dnaC (2) and other priming proteins in a “primosome” (3), thereby initiating φX174 DNA complementary strand synthesis (4, 5). It participates in φX RF replication (6), phase λ (7), and ColEI DNA replication (8). The dnaB protein from wild type strains has been purified to homogeneity (9, 10) and it was shown that the pure protein possesses a ribonucleoside triphosphatase activity (10, 11).

It is expected that the analysis of dnaB protein of *E. coli* dnaB mutants with well defined biological defects will help to clarify the role of this multifunctional enzyme. A variety of mutant dnaB or dnaB analog proteins have been isolated until now by affinity chromatography on immobilized ATP (12-14) using a φX174 DNA-dependent dnaB complementation assay. The dnaB protein binds to ATP-agarose and can be eluted by ATP or ADP. Following affinity chromatography, the recovery of dnaB activity often was 100% or even higher indicating a stabilizing and/or regenerating effect of ATP or ADP on the dnaB protein (12, 13). Therefore, we expected that the same nucleotides would also stabilize other mutant dnaB proteins whose isolation had been hindered so far because the proteins were found to be inactive in a dnaB complementation assay (12, 15). These are the dnaB proteins of (i) *E. coli* groPB, a subclass of dnaB mutants, which replicate certain λ P mutants but not λ wild type (16), and (ii) the initiation-defective *E. coli* dnaB252 mutant (12, 17).

As will be shown in this paper, dnaB activity, indeed, can be recovered in crude extracts from such dnaB mutant strains in the presence of ATP or ADP. Furthermore, it is found that the dnaB activity from an *E. coli* dnaBts mutant can be stabilized or even reactivated by the same nucleotides.

**EXPERIMENTAL PROCEDURES**

Materials and methods, unless otherwise indicated, were as previously described (14, 15, 18). *E. coli* strains used are listed in Tables I and II. Adenine ribonucleotides were from Boehringer. Stock solutions (0.5 mM) were prepared by dissolving the disodium salts of ATP, ADP, and AMP in bidistilled water and adjusting the pH to 7.6 by 1 M Tris-base. PEI-cellulose (20 × 20 cm plates) were from Schleicher and Schüll. Buffer A is 20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1 mM diethiothreitol, 1 mM MgCl₂, 50 mM NaCl, 10% (w/v) glycerol. It is supplemented with ATP, ADP, or AMP and/or additional MgCl₂ as indicated in Tables I and II.

**Cellular Crude Extract**—Bacteria were grown in TY medium and harvested at about 4 × 10⁸ cells/ml as described (13). The growth temperature was 30 °C if not otherwise noted. About 2 g of wet cell paste were obtained from 1 liter of culture. Bacteria were lysed by the lysozyme-spermidine procedure (19). The crude extract contained about 20 mg of protein/ml (about 50 mg of protein/g of wet cell paste) and was treated with streptomycin sulfate as described (13).

**Ammonium Sulfate Fraction**—Crude extract contained (final concentrations in parentheses) Tris-HCl, pH 7.5 (40 mM), EDTA (1 mM), dithiothreitol (15 mM), NaCl (70 mM), and residual amounts of sucrose (∼55%), spermidine (∼7 mM), and streptomycin sulfate (∼4%). To one part of extract, ammonium sulfate (0.243 g/ml) was slowly added and stirred for 30 min, and the suspension was kept overnight at 0 °C. The precipitate was collected by centrifugation (17,000 × g, 20 min), washed with Buffer A containing ammonium sulfate (0.263 g/ml), collected again by centrifugation, and dissolved in Buffer A. The material was dialyzed by rotary shaking against Buffer A for 4 h and repeated changes of Buffer A, centrifuged once again (17,000 × g, 10 min) in order to remove insoluble material, and frozen in liquid nitrogen. The other part of extract was supplemented with ATP, ADP, or AMP, and/or MgCl₂ (final concentrations are indicated in Tables I and II). Incubation was for 2 min at 0 °C before ammonium sulfate was added. All the following operations were as described above except that the nucleotide and MgCl₂ concentration in Buffer A was the same as at the beginning. The material (about 20 mg of protein/ml) was stored at −70 °C (Fraction I).

**Complementation Assay**—dnaB- and dnaC-complementing activity was determined by using Fraction I (100 to 150 μg of protein, prepared without ATP) from strain BT1071 dnaBts (13) and PC22 dnaCts (20), respectively. Fraction I from BT1071 was heated for 1 min at 37 °C in order to inactivate residual dnaBts protein. Assays were performed as described previously (13). One unit of dnaB- and dnaC-complementing activity represents the incorporation of 1 nmol of dTMP.

**Thin Layer Chromatography**—Turnover of ATP in Fraction I was determined by one-dimensional lithium chloride chromatography on PEI-cellulose layers as described (21). This system separates ATP, ADP, AMP, and adenine from each other and from the corresponding deamination products. Fraction I was deproteinized by the addition of an equal volume of trichloroacetic acid (20%). After centrifugation, the supernatant was extracted repeatedly with H₂O-saturated diethyl ether in order to remove the trichloroacetic acid. Ten to 60 μl of deproteinized Fraction I was applied onto a PEI-cellulose plate (20 × 20 cm). Adenosine, inosine, and the corresponding ribonucleotides were run in parallel as markers. Spots seen under the UV light were marked, scraped off the plate, and extracted by 0.1 M HCl at room temperature for 16 h. After centrifugation, the nucleosides and nucleotides were determined quantitatively by UV spectrophotometry.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

1 The abbreviation used is PEI, polyethyleneimine.
**Table I**

Recovery by ATP and ADP of dnaB activity from E. coli groPB dnaB mutants

| groPB mutant | Additions to Fraction I preparation | dnaB activity | |
|--------------|-----------------------------------|--------------|---|
|              | Nucleotide* | MgCl₂ | units/mg | Fraction |
| B⁺ | ATP | 10 | 0.44 |
| B612 | ATP | 10 | 0.45 |
|       | ATP | 10 | <0.02 |
| B534 | ATP | 10 | 0.76 |
|       | ADP | 2.5 | 0.28 |
|       | AMP | 2.5 | 0.28 |
| B558 | ATP | 10 | 0.25 |
|       | AMP | 2.5 | 0.28 |

* Nucleotides were present at 10 mM each (final concentration).

Table II

Effect of ATP on dnaBts and dnaCts proteins

| dnaBts | Growth temperature | ATP | MgCl₂ | dnaB⁺ | dnaC⁺ |
|--------|-------------------|-----|-------|-------|-------|
| BT1071 | 30°C | 10 | 10 | 0.51 | 1.00 |
| dnaB  | 10 | 5 | 0.43 | 0.61 | 1.15 |
|        | 1 | 0.24 | 0.34 | 0.62 |
| dnaB252 | 30°C | 10 | 10 | <0.02 | 0.21 |
| RS185 | 30°C | 10 | 0.93 | 0.28 | 0.60 |
| dnaB252 | 40°C | 10 | 0.63 | <0.05 | 0.10 |
|        | 1 | 0.48 | 0.0 | 0.0 |

* Micrograms of dnaB protein and units of dnaB and dnaC activity are expressed/mg of Fraction I, not measured.

**RESULTS**

Inactive groPB dnaB Protein Is Rendered Active by ATP and ADP—The dnaB protein of the E. coli groPB mutants B612, B534, B558, and B585 does not complement a dnaBts extract when Fraction I is prepared in Buffer A (1 mM MgCl₂, Table I), confirming earlier findings (15). However, when the preparations were done in the presence of ATP and MgCl₂ (10 mM each), dnaB activity was recovered (Table I). More detailed studies were undertaken with the mutant B612 which is temperature-sensitive in its groPB phenotype (16). Ten mM ATP alone (without MgCl₂) but not MgCl₂ alone (without ATP) was sufficient to yield dnaB activity (Table I). When 2 or 0.1 mM ATP (in the presence of 10 mM MgCl₂) was used, no activity was found. ADP is as effective as ATP, but AMP is not (Table I). The recovery of dnaB activity was not affected when Fraction I was prepared in the presence of 0.4 M NaCl in addition to ATP and MgCl₂. When a preparation of Fraction I with ATP and MgCl₂ (10 mM each) once did not yield dnaB activity, a subsequent supplementation with 10 mM ATP and repeated dialysis led to the recovery of activity (0.5 units dnaB/mg of Fraction I). In the presence of ATP and MgCl₂, dnaB activity of groPB612 (0.26 units/mg of Fraction I) was also recovered when the temperature of the growing culture was shifted to 40°C for 10 min before the bacteria were harvested. dnaB activity from the wild type strain (B⁺) was 1.8-fold higher when Fraction I was prepared in the presence of ATP and MgCl₂ (Table I).

Effect of ATP on dnaBts and dnaCts Protein Activity—The dnaBts protein of E. coli BT1071 is active in dnaB complementation when Fraction I is prepared in Buffer A. Again, the activity of the protein increased nearly 2-fold when the preparation was done in the presence of ATP and MgCl₂ (Table II). The dnaB protein of strain 252 does not complement a dnaBts extract as was found earlier (Ref. 12; S. Wickner, cited in Ref. 17). Even in the presence of ATP and MgCl₂ (10 mM each) no activity was recovered (Table II). However, when the dnaB252 mutant strain harbors the multicopy hybrid plasmid ColE1:dnaC, dnaB activity was found provided that ATP and MgCl₂ were present and the strain had been grown before at 30°C (Table II). Surprisingly, under conditions where the dnaB mutation is completely suppressed in vivo (i.e. at 40°C growth temperature, Ref. 23), no activity was found in vitro in the presence of ATP and MgCl₂, although dnaB protein was detected immunologically (Table II). The dnaC activity of strain BT1071 and RS185 roughly correlates with the corresponding dnaB activity, indicating complex formation between the two proteins (2). An attempt to stabilize the dnaCts protein of strain PC22 by the addition of ATP and MgCl₂ was, however, negative. Only the corresponding dnaB activity increased 2-fold under these conditions (Table II).

An increase in the dnaB activity by ATP and MgCl₂ is accompanied by a corresponding increase in the amount of dnaB protein recovered immunologically (Table II). Specific activities of 1300 to 2900 units/mg of dnaB protein were calculated for strain BT1071 and PC22, respectively. These values are similar to those found for the purified dnaB protein of other strains (13, 14). On the other hand, the specific activity of dnaB252 protein of strain RS185 is only about 300 (Table II).

Stabilization and Regeneration by ATP and ADP of dnaBts Protein Activity—Fraction I of strain BT1071 was prepared in Buffer A (=nucleotide), supplemented afterwards by ATP, ADP, or AMP, and incubated at 37°C. dnaB activity remained stable for more than 2 min in the presence of ATP and ADP, but not with AMP or without nucleotides (Fig. 1). Moreover, when an inactivated sample of Fraction I was supplemented by ATP and MgCl₂ and dialyzed for 4 h at 0°C, nearly 60% of the dnaB activity was recovered. MgCl₂ alone had no effect (Table III).

Turnover of ATP in Crude Extracts—The UV absorption of Fraction I in Buffer A + 10 mM ATP decreased considerably after 4 h of dialysis at 0°C against the same buffer. The decrease in optical density was more pronounced at 260 nm (up to 50%) than at 250 nm, indicating denaturation of aden-
ATP and ADP is too extensive, then no recovered. Certainly, this was the case when the starting concentration of ATP was 10 mM. On the contrary, if the degradation of ATP and ADP is much less than 10 mM, IMP (2.8 mM), and inosine (3.2 mM) as the major reaction products. The amount of these products varied considerably from experiment to experiment, but the concentration of ATP and ADP never exceeded 0.1 and 2 mM, respectively. These results suggest that the nucleotide concentration required for stabilization of dnaB protein activity may be much less than 10 mM. On the contrary, if the degradation of ATP and ADP is too extensive, then no dnaB activity may be recovered. Certainly, this was the case when 2 and 0.1 mM ATP was unsuccessfully used to recover groPB dnaB activity (see above). An extensive degradation of ATP also may have been the reason why we failed once (out of six preparations of Fraction I from groPB612) to recover dnaB activity even when the starting concentration of ATP was 10 mM.

**DISCUSSION**

Stabilization by ATP and ADP of dnaB protein activity in crude extracts can be exploited for the isolation of otherwise inactive mutant dnaB proteins. For example, the protein of *E. coli* groPB mutants exhibits dnaB complementing activity in the presence of ATP and ADP, respectively (Table I). Thus, the dnaB protein of groPB612 which in the absence of ATP was shown to be smaller than the corresponding dnaB+ protein (15) recovers the size (M, ~ 250,000) of the wild type protein in the presence of ATP. Obviously, ATP or ADP reenforces in vitro the formation of the dnaB multimer structure from the inactive and smaller, probably monomeric, form of the groPB612 protein. This interpretation is supported by the recent finding that in the presence of Mg+ ions both ATP and ADP can form a binary complex with dnaB+ protein (22). The fact that the groPB612 dnaB protein regains activity in the presence of ATP but without the addition of MgCl2 (Table I) must not mean that Mg+ ions are not required for dnaB protein stabilization, because Fraction I may still contain Mg+ ions derived from within the bacterial cells.

In contrast to the groPB mutants, ATP and MgCl2 are not sufficient to recover dnaB complementing activity from crude extracts of the dnaB252 mutant. Obviously, an excess of dnaC protein has to be present in addition (Table II). It remains to be shown whether on further purification the dnaB252 protein retains activity per se or only in the form of dnaB-dnaC complex. Neither dnaB nor dnaC activity was found in Fraction I from RS185 grown at 40 °C although dnaB protein was detected (Table II). Therefore, it appears questionable that suppression of the dnaB mutant by an excess of dnaC protein in vivo (23) is brought about simply by a stabilization of dnaB252 protein in a dnaB-dnaC complex.

ATP increases the dnaB activity in Fraction I from dnaB+ and dnaBts strains. The increase in activity is due to a corresponding increase in the amount of dnaB protein recovered. Thus, the presence of ATP during the preparation can improve the yield of dnaB protein. Stabilization of dnaB+ protein by ATP during the purification procedure has also been observed by others (24).

**Acknowledgments—**We are much indebted to I. Herskowitz, C. P. Georgopoulos, and J. A. Wechsler for supplying us with *E. coli* groPB and dnaB252 mutant strains. We are grateful to C. Michaelis for expert technical assistance.

**REFERENCES**

1. Kornberg, A. (1980) *DNA Replication*, W. H. Freeman & Company, San Francisco
2. Wickner, S., and Hurwitz, J. (1975) *Proc. Natl. Acad. Sci. U. S. A.* 72, 921-925
3. Aris, N., and Kornberg, A. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 69-73
4. Schekman, R., Weiner, J. H., Weiner, A., and Kornberg, A. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 5659-5665
5. Wickner, S., and Hurwitz, J. (1974) *Proc. Natl. Acad. Sci. U. S. A.* 71, 4120-4124
6. Aris, N., Aris, N., Shilomai, J., and Kornberg, A. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 3322-3326
7. Wickner, S. H. (1978) *Annu. Rev. Biochem.* 47, 1163-1191
8. Staudenbauer, W. L., Lanka, E., and Schuster, H. (1978) *Mol.**
dnaB Protein of Escherichia coli

9. Reha-Krantz, L. J., and Hurwitz, J. (1978) J. Biol. Chem. 253, 4043-4050
10. Ueda, K., McMacken, R., and Kornberg, A. (1978) J. Biol. Chem. 253, 261-269
11. Reha-Krantz, L. J., and Hurwitz, J. (1978) J. Biol. Chem. 253, 4051-4057
12. Lanka, E., Geschke, B., and Schuster, H. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 799-803
13. Lanka, E., Mikolajczyk, M., Schlicht, M., and Schuster, H. (1978) J. Biol. Chem. 253, 4746-4753
14. Lanka, E., Edelbluth, C., Schlicht, M., and Schuster, H. (1978) J. Biol. Chem. 253, 5847-5851
15. Günther, E., Lanka, E., Mikolajczyk, M., and Schuster, H. (1981) J. Biol. Chem. 256, 10712-10716
16. Georgopoulos, C. P., and Herskowitz, I. (1971) in The Bacteriophage λ (Hershey, A. D., ed) pp. 553-564, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Zyskind, J. W., and Smith, D. W. (1977) J. Bacteriol. 129, 1476-1486
18. Edelbluth, C., Lanka, E., von der Hude, W., Mikolajczyk, M., and Schuster, H. (1979) Eur. J. Biochem. 94, 427-435
19. Bouché, J. P., Zechel, K., and Kornberg, A. (1975) J. Biol. Chem. 250, 5995-6001
20. Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. A., and Barnoux, C. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 3150-3153
21. Randerath, K., and Randerath, E. (1967) Methods Enzymol. 12, 323-350
22. Arai, K., and Kornberg, A. (1981) J. Biol. Chem. 256, 5260-5266
23. Scialfani, R. A., and Wechsler, J. A. (1981) J. Bacteriol. 146, 418-421
24. Arai, K., Yasuda, S., and Kornberg, A. (1981) J. Biol. Chem. 256, 5247-5252
25. Wickner, S. H., Wickner, R. B., and Raetz, C. R. H. (1976) Biochem. Biophys. Res. Commun. 70, 389-396