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A DNA-Unwinding Protein Isolated from *Escherichia coli*: Its Interaction with DNA and with DNA Polymerases

(DNA–protein complex/DNA denaturation/DNA polymerase II)

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ABSTRACT **A DNA-unwinding protein has been purified to homogeneity from *E. coli*. This protein has a molecular weight of about 22,000, as judged by its electrophoretic mobility on polyacrylamide gels containing sodium dodecylsulfate, and it appears to be present in about 800 copies per log-phase cell. It binds tightly and cooperatively to single-stranded DNA, and much less tightly, if at all, to RNA or double-stranded DNA.**

Like the T4 gene-32 protein characterized previously, the *E. coli* DNA-unwinding protein depresses the melting temperature of double-stranded DNAs, with regions rich in A-T base-pairs being preferentially melted. The *E. coli* protein strongly stimulates *in vitro* DNA synthesis by *E. coli* DNA polymerase II on appropriate templates; however, no stimulation is found with purified polymerases I or III of *E. coli*, or with T4 DNA polymerase. In contrast, gene-32 protein stimulates only the T4 DNA polymerase in a parallel assay.

In vitro studies with purified DNA reveal that, at ionic strengths and temperatures in the physiological range, the double-helical DNA conformation is overwhelmingly stable relative to unpaired single-strands (1). *In vivo*, both DNA replication and genetic recombination would seem to demand a considerable loosening of this helical structure.

Recent work suggests that such DNA unwinding is facilitated within a cell by the presence of a special type of protein that drastically lowers helix stability by virtue of its tight cooperative binding to exposed single strands of DNA (2). The first protein of this type to be characterized was found in *Escherichia coli* infected with T4 bacteriophage, and is the product of T4 gene-32 (2–5). This "DNA-unwinding" protein is required for both genetic recombination (6, 7) and replication (8, 9) of T4 DNA. It stimulates the *in vitro* rate of DNA synthesis catalyzed by T4 DNA polymerase on single-stranded DNA templates by as much as 5- to 10-fold; in addition, a complex between the gene-32 protein and T4 DNA polymerase can be detected by physical methods (10).

We have examined several other biological systems for proteins that can unwind DNA *in vitro*, and which may therefore resemble T4 gene-32 protein in their function. By chromatography of DNA-free crude extracts on single-stranded DNA–cellulose columns (11), such proteins have recently been isolated from cells infected with filamentous bacteriophage (12, 13) and from calf-thymus tissue (G. Herrick and B. A., in preparation.) In this report, the isolation and characterization of a DNA-unwinding protein from *E. coli* is described, along with studies of its effect on *in vitro* DNA synthesis catalyzed by four different DNA polymerases.

MATERIALS AND METHODS

Basic techniques used for electron microscopy (5), DNA denaturation (2, 12), and polymerase assay (14) have been described; [^{3}H]TTP (2×10^5 cpm/nmole) was used throughout for measurements of DNA synthesis. *E. coli* polymerase II (fraction V, 270 units/mg) and T4 gene-32 protein were prepared by published procedures (14, 2), as was *E. coli* polymerase III (10,000 units/mg) (15). *E. coli* exonuclease III (180,000 units/mg) and DNA polymerase I (18,000 units/mg) were obtained from Dr. A. Kornberg; T4 DNA polymerase (3600 units/mg) was a generous gift from Dr. I. R. Lehman. (The units given are those originally defined for each enzyme.)

DNA from bacteriophages lambda and T7 was prepared by phenol extractions of the purified phage. Exonuclease III-treated DNA template (from phage T7) was prepared by incubation of 1 μmol of DNA (as nucleotide) with 2500 units of exonuclease III for 5 min at 30° in 4.5 ml of a buffer containing 0.09 M Tris-acetate (pH 8.2)–2 mM 2-mercaptoethanol–2 mM $MgCl_2$. The reaction was terminated by heating for 5 min at 65°. As judged by the maximum extent of DNA polymerase I-catalyzed repair at 30°, about 500 nucleotides were digested per phage equivalent of DNA. Exonuclease III- treated, nicked DNA template from calf-thymus was prepared as described (14); it contains many short single-stranded gaps, rather than the relatively long, single-stranded, termini expected for the T7 DNA.

RESULTS

Selection of E. coli Proteins That Bind Tightly to Single-Stranded DNA. In searching for possible candidates for a DNA-unwinding protein in *E. coli*, we have taken advantage of a technique discovered by G. Herrick, who found that the majority of proteins that bind to single-stranded DNA-cellulose can be eluted with a solution of 0.9 mg/ml of dextran sulfate (a highly charged anionic polysaccharide of average molecular weight 500,000). The small number of proteins left bound to the DNA–cellulose include some known DNA-unwinding proteins (G. Herrick and B. Alberts, in preparation). For *E. coli*, this fraction represents about 0.4% of the total soluble protein. Na dodecyl SO_4–polyacrylamide gel analysis shows major bands at positions corresponding to molecular weights of about 70,000, 22,000, and 10,000. These three protein classes, recovered by 2 M NaCl elution from DNA–cellulose, have been separated from each other

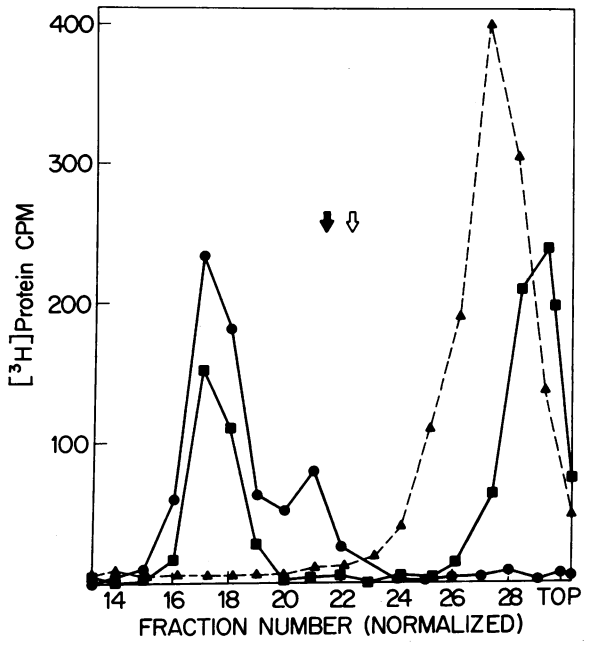


FIG. 1. Stoichiometry of binding of *E. coli* DNA-unwinding protein, determined by its cosedimentation with single-stranded nucleic acids. Linear 5–30% sucrose gradients (5 ml) were prepared in a buffer containing 0.05 M NaCl–0.01 M Tris·HCl (pH 7.4)–1 mM Na_3EDTA–1 mM 2-mercaptoethanol–100 μg/ml of bovine-serum albumin (Pentex)–10% glycerol. To the top of the gradient, a 0.2-ml sample was applied, containing 36 μg of [^{3}H]-leucine-labeled DNA-unwinding protein from *E. coli* plus either 8 μg of fd DNA (●——●), 2 μg of fd DNA (■——■), or 30 μg of R_{17} RNA (▲——▲). Centrifugation was for 90 min at 48,000 rpm in a Spinco SW50.1 rotor (4°). The *filled* and *empty arrows* indicate the positions expected for free fd DNA and R_{17} RNA, respectively, each of which sedimented in a single sharp peak. The presence of two peaks of [^{3}H]protein in the gradient with DNA excess reflects the strongly clustered binding found for this protein (see also Fig. 2*A*); note that the sedimentation rate of the free protein increases somewhat in the presence of R_{17} RNA, indicating a *weak* affinity for this nucleic acid. For calculation of stoichiometry, correction was made for a decreased recovery of DNA-bound relative to free [^{3}H]protein in control gradients. For the preparation of *E. coli* DNA-unwinding protein, *E. coli* D10 was grown at 32° in M-9 minimal medium containing 0.3% casein hydrolysate plus 1% glucose to 7×10^8 cells per ml, harvested, and stored at −20°. Cells (130 g) were broken by sonication in 300 ml of a buffer containing 0.05 M NaCl–0.01 M $MgCl_2$–2 mM $CaCl_2$–1 mM 2-mercaptoethanol–1 mM Na_3EDTA–0.02 M Tris·HCl (pH 7.4)–20 μg/ml of pancreatic deoxyribonuclease I (Worthington). The extract was incubated for 60 min at 10°, centrifuged at low speed to remove debris, clarified by centrifugation at 140,000 × *g* for 75 min, and dialyzed for 30 hr against several changes of 0.05 M NaCl–1 mM Na_3EDTA–1 mM 2-mercaptoethanol–0.02 M Tris (pH 7.4) (buffer A). After a second high-speed centrifugation to remove a light precipitate, the dialyzed extract was made 10% in glycerol and pumped at 50 ml/hr through a column containing 30-ml packed volume of denatured calf-thymus DNA–cellulose (about 1 mg of DNA per ml). Buffer A plus 10% glycerol was the basic buffer used for a 90-ml rinse and for elutions. The column was eluted at 50 ml per hr in the following steps: 150 ml of dextran sulfate 500 (Pharmacia; 0.87 mg/ml in rinse buffer), 90 ml of 0.05 M NaCl rinse buffer to wash out dextran sulfate, and 90 ml of 2.0 M NaCl rinse buffer. The peak fractions (30 ml) were dialyzed against a buffer containing 0.01 M NaCl–10% glycerol–1 mM Na_3EDTA–1 mM 2-mercaptoethanol–0.02 M Tris·HCl (pH 8.1) (buffer B) and applied to a 8 cm × 0.8 cm^2 column of DEAE-cellulose (Whatman DE-32). The column was washed and then eluted with a 40-ml linear NaCl gradient (0.01–0.5 M) in buffer B. 1.3-ml fractions were collected every 20 min. The 22,000-dalton protein appeared in four fractions, at a mean NaCl concentration of 0.20 M. This eluate was briefly dialyzed against 0.04 M potassium phosphate buffer (pH 7.0)–10% glycerol; the 22,000-dalton protein formed a precipitate that was collected, redissolved in buffer A containing 10% glycerol, and stored at −80°. With 50 μg of protein, only a single band was detected by Coomassie Blue staining after Na dodecyl SO_4–polyacrylamide gel electrophoresis or by gel isoelectric focusing in urea (isoelectric point about pH 7). In contrast, the 10,000- and 70,000-dalton protein classes each consist of several components; these two classes are recovered in the breakthrough of the DEAE-cellulose column and in the pH-7 supernatant, respectively. Because of its cooperative binding, most of the 22,000-dalton protein from *E. coli* no longer survives the rinse with dextran sulfate when the amount of *E. coli* extract applied to the single-stranded DNA-cellulose column is reduced 5-fold from that used here.

by standard fractionation techniques (see Fig. 1). When each protein was examined by electron microscopy, only the 22,000-dalton protein was observed to form complexes with single-stranded DNA similar to those seen (5) with T4 gene-32 protein; this protein will henceforth be referred to as the "*E. coli* DNA-unwinding protein."

Stoichiometry of Binding of the E. coli DNA-unwinding Protein to DNA. When Mg^{++} is added to single-stranded DNA at low ionic strengths (e.g., 1 mM K^+), the originally extended DNA chains fold upon themselves to form imperfect, intrastrand hairpin helices. This conformational transition is accompanied by an immediate 15% decrease in DNA absorbance at 260 nm (16). The T4 gene-32 protein keeps complexed single-stranded DNA in an extended conformation, preventing the immediate hypochromic shift normally seen upon Mg^{++} addition (unpublished results of B. A.). This fact provides the basis for a convenient general method for measuring the stoichiometry of the binding of an unwinding protein to single-stranded DNA. A constant amount of single-stranded DNA is mixed with different amounts of protein, and the instantaneous drop in absorbance on Mg^{++} addition is plotted for each protein–DNA ratio tested. At ratios equal to or exceeding saturation of the complex, no absorbance change should be observed. Results obtained for the 22,000-dalton *E. coli* protein indicate that the DNA should be saturated with protein at a weight ratio of protein to DNA of about 8 to 1. The complex would thus contain about one protein molecule (22,000 daltons) for every 8 nucleotides.

The stoichiometry of the complex was also determined by sucrose gradient sedimentation of a fixed quantity of [^{3}H]-leucine-labeled *E. coli* protein in the presence of various amounts of single-stranded DNA from phage fd (Fig. 1). At the lower concentration of DNA, two distinct peaks of radioactive protein were seen, one sedimenting rapidly with DNA, the second at the slow rate characteristic of the free protein. From the fraction of protein bound, a weight ratio of protein to DNA of about 8 to 1 was again determined. The fully saturated complex in Fig. 1 sediments only slightly faster than free DNA, a result that suggests that the complex has an extended conformation (see Fig. 2). The ^{3}H-labeled

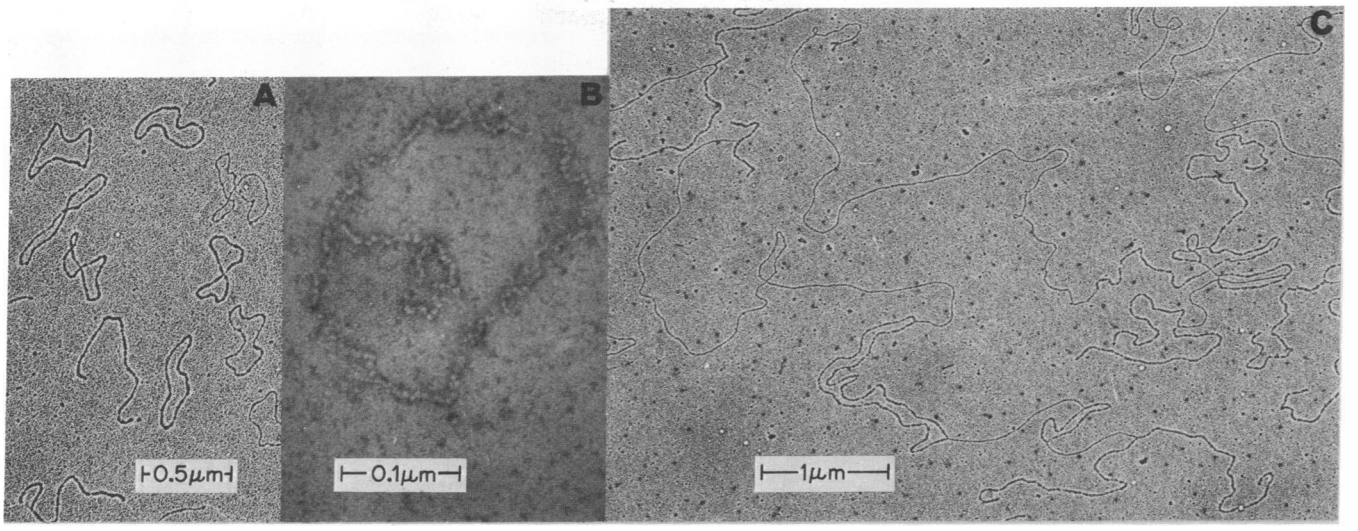


FIG. 2. Electron microscopy of complexes of *E. coli* DNA-unwinding protein with DNA. (*A*) Complexes of fd DNA and the *E. coli* protein prepared by the Kleinschmidt technique. Fd DNA (14 μg/ml) was incubated with *E. coli* DNA-unwinding protein (96 μg/ml) in 0.01 M potassium phosphate (pH 7.0) for 10 min at 30°. The sample was fixed with 0.01 M glutaraldehyde for 15 min at 37° and spread (5) with formamide and cytochrome *c*. (*B*) Complex of fd DNA and the *E. coli* protein prepared by negative staining. Fd DNA (14 μg/ml) was incubated with *E. coli* DNA-unwinding protein (120 μg/ml), fixed with glutaraldehyde, and diluted 50-fold into 0.01 M potassium phosphate (pH 5.0). The complexes were then absorbed to an air-glow-treated carbon film, which was then transferred to a drop of 2% uranyl formate and dried after excess stain was blotted off. (*C*) Bacteriophage lambda DNA partially denatured by *E. coli* DNA-unwinding protein. DNA from bacteriophage lambda (1.8 μg/ml) was incubated with the *E. coli* protein (53 μg/ml) in 0.01 M potassium phosphate (pH 7.0) for 10 min at 50° and fixed with 0.01 M glutaraldehyde for 15 min at 50°. The sample was spread with cytochrome *c* at a DNA concentration of 0.4 μg/ml.

E. coli protein did not bind to bacteriophage R17 RNA (Fig. 1) or to double-stranded T7 DNA (not shown) in parallel sucrose gradients, performed under identical conditions.

Structure of the DNA–Protein Complex. The approximate dimensions of the complex that the *E. coli* DNA-unwinding protein forms with circular, single-stranded fd DNA have been determined by electron microscopy. The complexes shown in Fig. 2*A* were prepared at a concentration of protein not sufficient to saturate all of the DNA. A strikingly nonrandom distribution of the protein is observed; some DNA molecules appear to be fully saturated with protein while others have little or no protein bound. Such strong clustering of protein molecules was also observed for T4 gene-32 protein complexes, and is indicative of a highly cooperative binding process (5).

The fully saturated structure has a circumference of 1.2 μm, which is about 35% smaller than that measured for the free fd DNA present. Since the fd DNA circle contains about 6600 nucleotides (17), the length increment along the circumference of the circle is only 1.8 Å per nucleotide. This spacing is much too small for a linear DNA chain, and the DNA in the complex must, therefore, either be in a broad helix or some other regularly folded conformation. This conformation must be rigidly imposed on the DNA by the protein, since the dimensions of the complex are unchanged when 1 mM spermine is added to the spreading mixture; in contrast, free DNA strands are folded into tight knot-like structures in the presence of spermine (5).

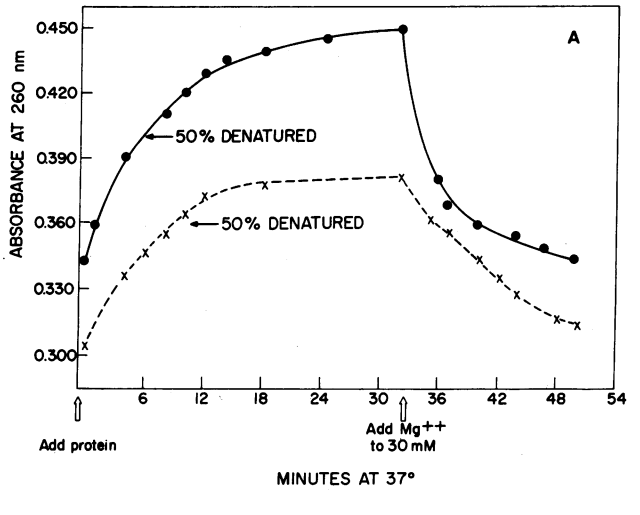
Fig. 2*B* shows a saturated complex of fd DNA and the *E. coli* DNA-unwinding protein, negatively stained with uranyl formate. Here detailed fine structure can be seen. The complex appears as a "beaded necklace" of 45–50-Å diameter, with "beads" spaced at about 60-Å intervals along the extended DNA single-strand. About 200 such protein spheres would be needed to cover a distance of 1.2 μm; this is roughly the number seen. However, with one protein monomer per 8 nucleotides (see above), there should be about 825 molecules of the 22,000-dalton protein bound to each fd DNA molecule. Thus, the "beads" seen in Fig. 2*B* probably represent an oligomeric form of the protein. In support of this view, the pure protein sediments on sucrose gradients in a sharp band at about 4.7 S in low salt buffers (corresponding to a molecular weight of 66,000, if spherical), demonstrating that the protein forms oligomers even without the DNA (unpublished results).

Protein-Catalyzed DNA Denaturation. The *E. coli* DNA-unwinding protein has a selective affinity for single-stranded over double-stranded DNA and, in addition, appears to completely cover the single-stranded DNA molecules to which it is bound. Therefore, this protein should facilitate the denaturation of double-stranded DNA (2, 12). Since single-stranded DNA is fully hyperchromic when complexed with the *E. coli* protein, such DNA denaturation can be monitored by the hyperchromic change at 260 nm that is characteristic of the DNA helix–coil transition. In Fig. 3*A*, absorbance measurements have been used to demonstrate that T4 DNA can be denatured in the presence of excess *E. coli* unwinding protein at low ionic strengths. As expected, the extent of denaturation attainable increases with the amount of protein added. Addition of 0.03 M Mg^{++} to the partially denatured molecules causes the unwound strands to rapidly rejoin, since Mg^{++} increases the T_m of the DNA and shifts the equilibrium back toward the native form (see ref. 2).

The rapidity of helix reformation in Fig. 3*A* must reflect

the fact that the strands had not been completely separated, since attempts to renature *free* single-stranded DNA covered with *E. coli* unwinding protein under these conditions have not yet been successful (unpublished results of N. S.).

When double-stranded bacteriophage λ DNA is incubated with the *E. coli* unwinding protein and fixed with glutaraldehyde, regions of local DNA denaturation are readily recognized in the electron microscope as bifurcations in its otherwise linear structure (Fig. 2*C*). When the locations of these denatured regions are tabulated, a DNA denaturation map is obtained analogous to that described by Inman and Schnös for heat and alkali denaturations (18). This map is presented in Fig. 3*B;* comparison with Inman's data reveals that the A–T-rich regions of λ DNA are preferentially opened. This result would be expected with a DNA-unwinding protein lacking sequence specificity, since A–T base pairs are weaker than G–C base-pairs, and are, therefore, more easily opened.



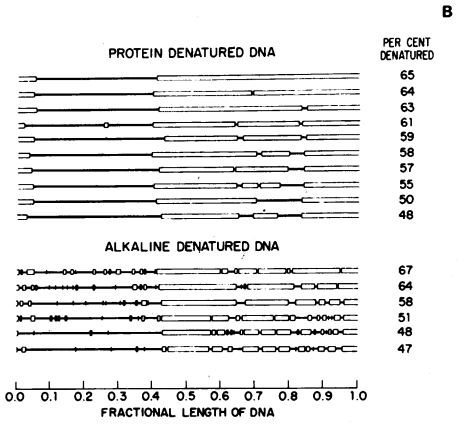


FIG. 3. DNA denaturation catalyzed by *E. coli* DNA-unwinding protein. (*A*) Native T4 DNA (at a concentration of 15 μg/ml) in 1 mM KCl–2 mM Tris·HCl (pH 8.1)–0.1 mM Na_3-EDTA was added to microcuvettes at 37° in a thermostatted compartment of a Gilford spectrophotometer. At time zero, a concentrated stock of unwinding protein was added to final concentrations of 135 μg/ml (×– – –×) or 270 μg/ml (●——●). At the indicated time, $MgCl_2$ was added to a concentration of 0.03 M to both reaction mixtures. The melting temperature of the T4 DNA alone in this buffer is 55°. (*B*) Partial denaturation maps of lambda DNA. The upper set of denaturation maps was obtained from length measurements on DNA partially denatured by *E. coli* DNA-unwinding protein, as shown in Fig. 2*C* (correction was made for a 35% decrease in length of denatured sections). The lower set of denaturation maps shows lambda DNA denatured to a comparable extent by alkali, by a modification of the procedure described by Inman and Schnös (18, 19). Denatured regions are represented by the *paired lines,* with molecules oriented with their AT-rich half on the *right.* Note that the denaturation pattern obtained with the *E. coli* protein does not show as much fine structure as observed with alkaline denaturation. Therefore, it seems that the *E. coli* protein has a strong preference for expanding denaturation loops, as opposed to initiating new ones, probably as a result of its highly cooperative DNA binding.

Stimulation of E. coli DNA Polymerase II. Brief digestion of "nicked" double-stranded DNA with exonuclease III produces short gaps, which are readily repaired by *E. coli* DNA polymerase II (14, 20). The *E. coli* DNA-unwinding protein has no effect on either the rate or extent of this reaction. Long single-stranded regions, produced by exonuclease III-degradation of intact double-stranded DNA, serve only poorly as templates for polymerase II (20, 21). The addition of *E. coli* unwinding protein to these templates stimulates the rate of synthesis by polymerase II almost 10-fold. The rate of synthesis observed in the presence of different amounts of unwinding protein is shown in Fig. 4*A*. The amount of unwinding protein required for maximal activity is proportional to the amount of single-stranded template present; higher levels are inhibitory. A similar effect of T4 gene-32 protein on the T4 DNA polymerase reaction has been reported (10). Controls show that heat inactivation of the unwinding protein subsequent to its incubation with template abolishes its stimulatory activity; thus, the unwinding protein does not irreversibly affect the DNA.

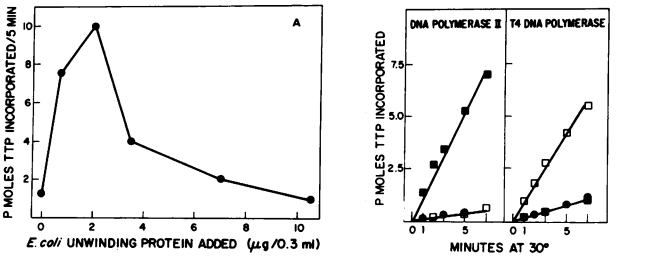


FIG. 4. (*A*) Effect of unwinding protein concentration on DNA polymerase II activity. The reaction mixture (0.3 ml) contained 20 μmol Tris-acetate (pH 7.4); 2 μmol $MgCl_2$; 1 μmol 2-mercaptoethanol; 10 nmol (each) of dCTP, dGTP, and dATP, and 10 nmol of [^{3}H]TTP; 11 nmol (of nucleotide) of exonuclease III-treated T7 DNA, and unwinding protein (0.7–10.5 μg). The reaction was initiated by the addition of DNA polymerase II (0.02 units). Incubation was for 5 min at 30°. The reaction was terminated by the addition of 2.5 ml of 5% trichloroacetic acid, and the acid-insoluble radioactivity was collected on Millipore filters and counted. (*B*) Specificity of stimulation of DNA polymerase by unwinding protein. The reaction mixture (0.6 ml) contained 40 μmol Tris-acetate (pH 7.4); 4 μmol $MgCl_2$; 2 μmol 2-mercaptoethanol; 20 nmol (each) of dCTP, dGTP, and dATP, 20 nmol [^{3}H]TTP; 22 nmol (of nucleotide) of exonuclease III-treated λ DNA; and either *E. coli* unwinding protein (■——■), T4 gene-32 protein (□——□), or no unwinding protein (●——●). The optimal concentration for each protein was used, determined as in Fig. 1. The reaction mixture was brought to 30° and synthesis was initiated by the addition of *E. coli* DNA polymerase II (50 units) or T4 DNA polymerase (0.4 units), as indicated. Aliquots (0.1 ml) were withdrawn at the times indicated, and the acid-insoluble radioactivity was determined.

In order to examine the specificity of the polymerase stimulations observed, comparable amounts of the T4 and *E. coli* DNA-unwinding proteins were tested for their effect on DNA polymerase I, II, and III from *E. coli* and on T4 DNA polymerase. As template, exonuclease III-degraded DNA from bacteriophage λ was used. The only stimulations observed were with polymerase II for the *E. coli* DNA unwinding protein, and with T4 polymerase for the T4 gene-32 protein. These results are shown in Fig. 4*B*. No stimulations were observed with either polymerase I or polymerase III (not shown).

DISCUSSION

The physical basis for the specificity of polymerase stimulation seen in Fig. 4*B* may lie in the very different geometry of the *E. coli* and T4 DNA-unwinding protein complexes with DNA: as judged by electron microscopy, these two otherwise very similar proteins form complexes with spacings of about 1.8 Å per nucleotide (Fig. 2*A*) and 4.6 Å per nucleotide (5), respectively. The fact that stimulation was observed only between *homologous* protein pairs suggests that these polymerases and unwinding proteins function together *in vivo*, as well as *in vitro*.

We estimate that there are about 800 molecules of the *E. coli* DNA-unwinding protein present in a rapidly-dividing *E. coli* (0.1% of the total protein); since each cell has about six replication forks, this is roughly the same number of molecules per replication fork as estimated previously for gene-32 protein in T4 (2). By analogy with the presumed role of T4 gene-32 protein in replication (4), the *E. coli* protein might thus be required in stoichiometric amounts for the proper alignment of DNA template strands in *E. coli* replication forks.

On the other hand, there is no evidence that *E. coli* polymerase II, the enzyme that was stimulated by the *E. coli* unwinding protein *in vitro*, is involved in replicative synthesis; genetic evidence demonstrates rather that DNA polymerase III is essential for *E. coli* DNA replication (22, 23). The purified polymerase III was not stimulated by our protein preparation; however, different conditions may be needed in order to demonstrate such stimulation, as suggested by recent results of Schekman *et al.* (24), who find that DNA synthesis on phage M13 DNA templates in fractionated extracts requires the 22,000-dalton unwinding protein from *E. coli* in addition to polymerase III.

Studies with the known *E. coli* mutants temperature-sensitive for DNA synthesis have not yet revealed any alteration of the *E. coli* DNA-unwinding protein present in these strains (unpublished results of S. Imada and N. S.). One might also expect that a DNA-unwinding protein might be required for *E. coli* genetic recombination: preliminary results suggest that the 22,000-dalton protein is normal in *rec* A mutants. It should be kept in mind that more than one DNA unwinding protein may exist in *E. coli*, and these proteins could have separate roles in the different genetic processes.

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1. Marmur, J. & Doty, P. (1962) *J. Mol. Biol.* **5**, 109–115.
2. Alberts, B. & Frey, L. (1970) *Nature* **227**, 1313–1318.
3. Alberts, B, Amodio, F. Jenkins, M., Gutmann, E. & Ferris, F. *Cold Spring Harbor Symp. Quant. Biol.* **33**, 289–305.
4. Alberts, B. (1971) in *Nucleic Acid-Protein Interactions and Nucleic Acid Synthesis in Viral Infection*, eds. Ribbons, D. W., Woessner, J. F. & Schultz, J. (North-Holland, Amsterdam), pp. 128–143.
5. Delius, H., Mantell, N. J. & Alberts, B. (1972) *J. Mol. Biol.* **67**, 341–351.
6. Tomizawa, J., Anraku, N. & Iwama, Y. (1966) *J. Mol. Biol.* **21**, 247–284.
7. Broker, T. R. & Lehman, I. R. (1971) *J. Mol. Biol.* **60**, 131–149.
8. Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, R., Chevallez, R., Edgar, R. S., Susman, M., Denhardt, G. H. & Lielausis, A. (1963) *Cold Spring Harbor Symp. Quant. Biol.* **28**, 375–394.
9. Kozinski, A. & Felgenhauer, (1967) *J. Virol.* **1**, 1193–1202.
10. Huberman, J., Kornberg, A. & Alberts, B. (1971) *J. Mol. Biol.* **62**, 39–52.
11. Alberts, B. & Herrick, G. (1971) "Nucleic Acids," in *Methods in Enzymology*, eds. Grossman, L. & Moldave, M. (academic Press, New York), Vol XXII, pp. 198–217.
12. Alberts, B., Frey, L. & Delius, H. (1972) *J. Mol. Biol.* **68**, 138–148.
13. Oey, J. & Knippers, R. (1972) *J. Mol. Biol.* **68**, 125–137.
14. Kornberg, T. & Gefter, M. L. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 761–764.
15. Kornberg, T. & Gefter, M. L. (1972) *J. Biol. Chem.* **247**, 5369–5375.
16. Studier, F. W. (1969) *J. Mol. Biol.* **41**, 189–198.
17. Marvin, D. A. & Hohn, B. (1969) *Bacteriol. Rev.* **33**, 172–209.
18. Inman, R. B. & Schnös, M. (1970) *J. Mol. Biol.* **49**, 93–98.
19. Mulder, C. & Delius, H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3215–3219.
20. Gefter, M. L., Molineux, I. J., Kornberg, T. & Khorana, H. G. (1972) *J. Biol. Chem.* **247**, 3311–3326.
21. Wickner, R. B., Ginsberg, B., Berkower, I. & Hurwitz, J. (1972) *J. Biol. Chem.* **247**, 489–497.
22. Gefter, M. L., Hirota, H., Kornberg, T., Wechsler, J. A. & Barnoux, C. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3150–3153.
23. Nusslein, V., Otto, B., Bonhoeffer, F. & Schaller, H. (1971) *Nature* **234**, 285–286.
24. Schekman, R., Wickner, W., Westergaard, O., Brutlag, D., Geider, K., Bertsch, L. L. & Kornberg. A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2691–2695.