Single-stranded DNA Structure and DNA Polymerase Activity in the Presence of Nucleic Acid Helix-unwinding Proteins from Calf Thymus*

GLENN HERRICK,‡ HAJO DELIUS,§ AND BRUCE ALBERTS¶

From the Department of Biochemical Sciences, Princeton University, Princeton, New Jersey 08540 and the Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

In the preceding articles we have described the isolation and some of the properties of two calf thymus proteins which bind selectively to single-stranded DNA and which appear analogous to previously isolated prokaryotic DNA-unwinding proteins. In the present work we demonstrate two further points of analogy. First, both the calf UP1 and the high salt eluting proteins form protein-rich complexes with single-stranded DNA, and hold this DNA in a rigid, extended conformation. Second, these proteins stimulate the calf thymus DNA polymerase-α; phage T4 gene 32-protein does not. The stimulation of a homologous DNA polymerase is characteristic of several prokaryotic DNA-unwinding proteins and is assumed to reflect their in vivo role in DNA synthesis.

Bacteriophage T4 gene 32-protein is known from genetic analysis to be required for T4 DNA replication (1). In vitro experiments show that this DNA-unwinding protein specifically stimulates the T4 DNA polymerase, and that there is a weak interaction between 32-protein and polymerase in the absence of DNA (2). Similar stimulations of homologous DNA polymerases have been demonstrated with the Escherichia coli and T7 DNA-unwinding proteins (3-6). The 32-protein and E. coli unwinding proteins have been shown to form tight complexes with single-stranded DNA in which all sequences of the DNA are bound and the DNA is held in an extended, regular conformation; however, the two complexes differ in contour length per nucleotide, and in composition (3, 7). The specific relationship between homologous polymerases and unwinding proteins could reflect differences in the conformation of the template strand associated with unwinding protein, further specificity being derived from direct unwinding protein-polymerase interactions.

In this report, we present properties of the single-stranded DNA complexes which are formed with the calf thymus helix-unwinding proteins described in the two preceding articles (8, 9). Both UP1 and the high salt eluting protein fraction are shown to stimulate in vitro DNA synthesis by the calf thymus DNA polymerase-α, thus extending the analogy set forth in the preceding articles between these calf proteins and prokaryotic DNA-unwinding proteins. Preliminary reports of this work have been presented (13, 14).

MATERIALS AND METHODS

The calf thymus single-strand-specific, dextran sulfate-resistant DNA-binding proteins were isolated as described in an accompanying article (8). By gel electrophoresis in sodium dodecyl sulfate, UP1 was judged >99% pure; the high salt eluting protein fraction contained 60% 35,000 dalton protein, along with minor proteins of higher molecular weight (for electrophoretic analyses see Refs. 8 and 10). The calf proteins showed neither DNA polymerase activity nor DNA nuclease activity. (Per µg of protein, less than 3 pmol of acid-soluble nucleotides were released from an equal mixture of radioactive Escherichia coli native and denatured DNAs during 45 min at 30° under DNA polymerase-α assay conditions.) T4 bacteriophage gene 32-protein was purified free of exonuclease. The calf thymus DNA polymerase-α (12), a gift of Drs. L. Chang and F. J. Bollum (University of Kentucky), had a specific activity of 1 µmol/hour/mg (assayed at 35° on activated calf thymus DNA). E. coli exonuclease III was the gift of Dr. I. R. Lehman (Stanford University). Bacteriophage fd DNA was prepared according to Ref. 16. SV40 DNA was obtained from Dr. H. Westphal (Cold Spring Harbor Laboratory). Synthetic DNA polymers were prepared by Drs. L. Chang and F. J. Bollum according to Ref. 17.

RESULTS

Structure of Complexes: Electron Microscopy—Techniques have been developed which optimize the visualization of the DNA polymerases (May, 1975); it has been used recently in the literature (10, 11), and DNA polymerase-α represents the 6 to 8 S polymerase of Yoneda and Bollum (12). This nomenclature system has been recently published (12a).

* This research was supported by grants from the National Institutes of Health and the American Cancer Society at Princeton and by grants from the National Institutes of Health at Cold Spring Harbor.
‡ Predoctoral Fellow of the National Science Foundation. Present address, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80302
§ Present address, Max Planck Institute, Munich, West Germany.
¶ To whom reprint requests should be addressed.
1 Abbreviation used is: UP1, calf nucleic acid helix-unwinding protein 1. The DNA polymerase nomenclature used in this paper is as tentatively adopted at the Asilomar Conference on Eukaryotic DNA

2142
glutaraldehyde-fixed DNA complexes of T4 gene 32-protein and of Escherichia coli DNA-unwinding protein (3, 7). Shown in Fig. 1 are analogous electron micrographs of single-stranded fd bacteriophage DNA circles, associated with an excess of either the calf high salt eluting proteins (Fig. 1, a and b) or the calf UP1 (Fig. 1c). The fixed complexes are extended circles, apparently uniformly coated with protein. These protein-covered DNA strands are not as thick as those seen with prokaryotic DNA-unwinding proteins, and thus are more difficult to distinguish from the naked single-stranded DNA.

The fd DNA contour length in the complexes formed with either of these proteins is about 2.4 μm, about 0.4 μm longer than that of the naked DNA spread in the same conditions. For comparison, the contour length of fd DNA associated with T4 32-protein was found to be about 1.1 μm longer, while the E. coli protein complex was about 0.6 μm shorter, than the naked DNA (3, 7).

Note that the fixed complexes in Fig. 1, b and c remain extended even when spread in the presence of 10 mM Mg**⁺, 1 mM spermine, conditions which induce a drastic intrastand folding in naked, single-stranded DNA (7). (The complexes contract slightly and adopt a somewhat irregular shape, possibly as a consequence of incomplete fixation.) Thus, as in the case of the prokaryotic DNA-unwinding proteins, the associated DNA appears to be rigidly held in a conformation that does not permit intrastrand DNA hairpin helix formation. This is consistent with the disappearance of ultraviolet hypochromicity, normally associated with single-stranded DNA secondary structure, upon complex formation (9).

As expected from their DNA melting properties (9), both the high salt eluting proteins and UP1 induce denaturation bubbles in SV40 supercoiled, double-helical DNA. This result with UP1 is shown in Fig. 1d.

Structure of UP1 Complex: Sedimentation Studies—Fig. 2a shows an experiment in which a fixed amount of ^H-labeled, single-stranded fd DNA was sedimentoated in the presence of increasing amounts of UP1. Initially, as shown in the inset of Fig. 1A, the S value of the DNA in the complex increases linearly with the amount of protein added. By this criterion, saturation is reached at a protein:DNA weight ratio of about 10:1, where the complex sediments about 3 times faster than the free DNA (see also Fig. 2B).

Direct determination of the stoichiometry of the saturated DNA-protein complex was carried out on sedimented complexes formed with excess UP1 (Fig. 2B, input protein:DNA weight ratios of 13:1 and 24:1). The protein:DNA weight ratio in the peaks was found to be 12:1 and 9:1:1, respectively. Taking the weight ratio to be 10:1, we calculate that each UP1 molecule would occupy a single-stranded DNA site about seven nucleotides long in the saturated complex. The protein:DNA weight ratio is in the same range as that obtained by similar techniques with prokaryotic “DNA-unwinding” proteins: 12:1 for the phage T4 gene 32-protein (19), 7:1 for the Ff gene 5 protein (16, 18), and 8:1 for the E. coli DNA-unwinding protein (3).

Fig. 2B also contains a sedimentation profile obtained under identical conditions for fd DNA saturated with the T4 gene 32-protein. Both sedimentation and electron microscopy (7, 20) suggest that this complex adopts a greatly extended conformation with a high frictional coefficient. The UP1 complex sediments even slower than the 32-protein complex. In fact, the frictional coefficients of the two complexes are nearly identical, the faster sedimentation of the 32-protein complex being a consequence of its somewhat larger mass. Thus, the sedimentation data provide independent evidence that the UP1 complex with single-strand DNA is highly extended in solution.

Stimulation of Calf Thymus DNA Polymerase-α—The effect of UP1 on the activity of the calf thymus DNA polymerase-α was tested, using as the template native DNA partially degraded with E. coli exonuclease III. With a quantity of UP1 approximately sufficient to cover the single-stranded DNA “tails” on this template, the rate of nucleotide incorporation was stimulated 10-fold (Fig. 3); higher amounts of UP1 inhibit the polymerase. In both respects, these results parallel those with prokaryotic unwinding proteins (2, 3). Note that no stimulation of the calf DNA polymerase was obtained with additions of T4 gene 32-protein (Fig. 3), showing that mere removal of secondary structure from the template is not sufficient for the DNA polymerase stimulation.

Slight (<2 fold) stimulations of this polymerase by UP1 have been observed both using heat-denatured DNA templates, and several synthetic Chang-Bollum “hook polymers” (17). In addition, strong stimulation (>5-fold) was found for oligo(dG)-primed synthesis of poly(dG) on a poly(dC) template (15).

For polymerase stimulation, a UP1 fraction composed primarily of the most basic subspecies (as judged by isoelectric focusing (8)) is the most effective. For example, in experiments using the exonuclease III-degraded DNA template, the optimal protein to single-stranded DNA weight ratio for stimulation was about 5:1 for the most basic UP1 subspecies, as against 27:1 for the most acidic subspecies. The basic subspecies is also the one which binds to DNA the most tightly (8).

The high salt eluting proteins behave similarly to UP1 in many respects (8, 9, and Fig. 1). Preliminary studies using the poly(dC)-oligo(dG) template-primer reveal this fraction to be even more efficient than the basic subspecies of UP1 in stimulating the calf DNA polymerase-α (15). Further characterization of this protein fraction must await the development of better purification procedures for its several components. Three reports of mammalian DNA polymerase-stimulating proteins have appeared (21, 22, 23), but details necessary for comparison with our results have not been published.

The third major calf DNA-binding protein fraction obtained by our isolation procedures does not appear to be a helix-unwinding protein by several criteria. Designated as the “33,000 dalton, low salt eluting protein” (8), it formed no detectable complex with fd DNA as judged by the electron microscopic criterion applied to the two other calf protein fractions (Fig. 1). Moreover, it is not capable of strong induction of DNA melting (9). Over a range of concentrations it has a marked inhibitory effect on the calf DNA polymerase-α.

S. Imada, G. Herrick, and F. Bollum, unpublished results.
FIG. 1. Electron microscopy of DNA-protein complexes containing single-strand-specific, dextran sulfate-resistant DNA-binding proteins from calf thymus. a, complex between fd single-stranded DNA and the high salt eluting proteins. The fd DNA (7 μg/ml) was incubated with the protein (160 μg/ml) for 10 min at 37° in 10 mM potassium phosphate/1 mM Na₂EDTA, pH 7. As detailed in Ref. 8, the protein
in tests using poly(dC)-oligo(dG); at a protein to single-stranded DNA weight ratio of 3.5:1, the polymerase was inhibited by 70% (15).

A second type of DNA-dependent DNA polymerase is found in mammalian cells (DNA polymerase-β); it is found in the chromatin fraction and sediments at about 3.3 S (24). We attempted to determine whether UP1 is also capable of stimulating polymerase-β. Using such a polymerase from rabbit bone marrow (24), we were unable to obtain any stimulation by UP1 when added over a wide range of concentrations both with our normal conditions (Fig. 3) and with the high pH conditions optimal for polymerase-β. These negative results could be attributed to differences between the calf thymus and rabbit bone marrow polymerases. However, the

fraction used was about 60% 33,000 dalton material, with the remainder consisting of small amounts of several proteins of higher molecular weights (no significant quantity of UP1 was present). After fixation with 10 mM glutaraldehyde for 15 min at 37°C, 3.5 µg/ml of free fd DNA was added and the sample was spread in formamide after a dilution to a final DNA concentration of 1 µg/ml as described earlier (7). b, effect of magnesium on the fixed complex. The sample was prepared with 130 pg/ml of pure UP1, consisting primarily of the most basic subspecies (8); comparable complexes have been observed with other subspecies of this protein. Free UP1, present after fixation, seems to inhibit by 70% (15). Stimulation by UP1 when added over a wide range of concentrations both with our normal conditions (Fig. 3) and with the high pH conditions optimal for polymerase-β. These negative results could be attributed to differences between the calf thymus and rabbit bone marrow polymerases. However, the

![Diagram](http://www.jbc.org/)
two enzymes appear to be quite similar (25), and more recently, pure calf polymerase-β has been tested for stimulation by calf UP1. Using the poly(dC)-oligo(dG) template-primer, no stimulation was found, although the polymerase-α stimulation was confirmed.

**DISCUSSION**

The stimulation of the polymerase-α by the calf proteins may merely result from the fact that the associated template is freed of inhibiting hairpin helices; however, the failure of T4 gene 32-protein to stimulate suggests that the calf proteins may in addition hold the template bases in a specific conformation optimal for rapid calf polymerase action. Moreover, in the bacteriophage T4 system, the gene 32-protein and the DNA polymerase have been demonstrated to form a weak complex in the absence of DNA (2). Such protein-protein interactions might also contribute to the specific stimulations seen in the calf and other systems (2-6).

Both the calf helix-unwinding proteins and the calf DNA polymerase-α are found predominantly in the chromatin-free, soluble fraction of extracts (8, 12). Various experiments (26-32) suggest that this DNA polymerase is involved in nuclear DNA replication, and it is thus possible that one or both of the two calf thymus helix-unwinding proteins are also involved in DNA replication. Note that there is a close analogy between the properties of these two proteins and the properties of the T4 gene 32-protein and the E. coli "DNA-unwinding protein," both of which appear to be involved in prokaryotic DNA synthesis. (a) All four proteins can be isolated as single-strand-specific, dextran sulfate-resistant DNA-binding proteins; (b) while all have nonbasic isoelectric points, their DNA binding is salt-sensitive and thus relies in part on ionic interactions with the DNA phosphates; (c) these proteins all hold single-stranded DNA in an extended complex which is unique and protein-rich; (d) these complexes show nearly complete DNA hyperchromicity, indicating that the bases are held in a nonstacked conformation; (e) by virtue of the above properties and their relative lack of affinity for native DNA, these proteins all have the ability to denature native DNA; (f) all of these proteins stimulate a homologous DNA polymerase.

On the other hand, there are two major differences between 32-protein and the calf proteins. First, as is true for the E. coli protein (3), no catalysis of DNA renaturation has been demonstrated. Second, the calf proteins stimulate a homologous DNA polymerase and are required. Regardless of their exact role, it is clear that proteins which closely resemble the prokaryotic DNA-unwinding proteins are widespread in nature, suggesting that they perform vital roles in biological processes. In this connection, we call attention to the recent report of a fungal DNA-unwinding protein which stimulates its homologous DNA polymerase (34).

**Acknowledgments—**We thank Mrs. Judy Goldberg and Dr. Sumi Imada for help with some of the polymerase experiments, and Drs. Lucy Chang and Fred Bollum for gifts of enzymes and templates and for their hospitality and aid during a visit of one of us (G. H.) to the University of Kentucky.

**REFERENCES**

1. Kozinski, A. W. & Felgenhauer, Z. Z. (1967) J. Virol. 1, 1193-1202
2. Huberman, J. A., Kornberg, A. & Alberts, B. M. (1971) J. Mol. Biol. 63, 39-52
3. Sigal, N., Delius, H., Kornberg, T., Gefter, M. L. & Alberts, B. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 3537-3541
4. Molineux, I. J., Friedman, S. & Gefter, M. L. (1974) J. Biol. Chem. 249, 6990-6998
5. Reuben, R. C. & Gefter, M. L. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 1456-1460
6. Scherzinger, E., Litfin, F. & Jost, E. (1973) Mol. Gen. Genet. 123, 247-262
7. Delius, H., Mantelli, N. & Alberts, B. (1972) J. Mol. Biol. 61, 341-350
8. Herrick, G. & Alberts, B. (1976) J. Biol. Chem. 251, 2124-2132
9. Herrick, G. & Alberts, B. (1976) J. Biol. Chem. 251, 2133-2141
10. Spadari, S. & Weissbach, A. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 503-507
11. Bollum, F. J. (1975) Proc. Nati. Acad. Sci. U. S. A. 72, 1486-1500
12. Weissbach, A., Baltimore, D., Bollum, F., Gallo, R. & Korn, D. (1975) Science 190, 401-402
13. Alberts, B., Herrick, G., Sigal, N., Frey, L. & Delius, H. (1971) Fed. Proc. 30, 1036 Abstr.
14. Herrick, G. & Alberts, B. (1973) Fed. Proc. 32, 497 Abstr.
15. Herrick, G. (1973) Ph.D. thesis, Princeton University
16. Alberts, B., Frey, L. & Delius, H. (1972) J. Mol. Biol. 68, 139-152
17. Chang, L. M. S. (1973) J. Biol. Chem. 248, 6983-6992
18. Oey, J. C. & Knippers, R. (1972) J. Mol. Biol. 65, 125-138
19. Alberts, B. (1971) in *Nucleic Acid-Protein Interactions* (Robbins, D. W., Woessner, J. & Schultz, J., eds) pp. 128-143, North Holland Publishing Co., Amsterdam & London
20. Alberts, B. M. & Frey, L. (1970) Nature 227, 1313-1318
21. Chiu, J. & Sung, C. (1971) Biochim. Biophys. Acta 251, 54-60
22. Probst, G. S., Stalker, D. M., Mosbaugh, D. W. & Meyer, R. R. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1171-1174
23. Wang, E. C. & Furth, J. J. (1974) Fed. Proc. 33, 1493 Abstr.
24. Chang, L. M. S. & Bollum, F. J. (1972) Biochemistry 11, 1264-1272
25. Chang, L. M. S. (1972) J. Biol. Chem. 247, 3793-3795
26. Ishumura, Y., Ono, T. & Morris, H. P. (1968) Cancer Res. 28, 2466-2476
27. Lech, L. A. & Agarwal, S. S. (1971) Exp. Cell Res. 66, 299-304
28. Chang, L. M. S. & Bollum, F. J. (1972) J. Biol. Chem. 247, 7948-7950
29. Chang, L. M. S., Brown, M. & Bollum, F. J. (1973) J. Mol. Biol. 74, 1-8
30. Lynch, W. E. & Lieberman, I. (1973) Biochim. Biophys. Res. Commun. 52, 843-849
31. Baril, E. F., Jenkins, M. D., Brown, E. O., Laszlo, J. & Morris, H. P. (1973) Cancer Res. 33, 1187-1193
32. Spadari, S. & Weissbach, A. (1974) J. Mol. Biol. 86, 11-20
33. Tomizawa, J., Annaka, K. & Iswana, Y. (1966) J. Mol. Biol. 21, 247-253
34. Banks, G. R. & Saposnik, A. (1973) J. Mol. Biol. 93, 63-71
Single-stranded DNA structure and DNA polymerase activity in the presence of nucleic acid helix-unwinding proteins from calf thymus.
G Herrick, H Delius and B Alberts

J. Biol. Chem. 1976, 251:2142-2146.

Access the most updated version of this article at http://www.jbc.org/content/251/7/2142

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/7/2142.full.html#ref-list-1