Escherichia coli DNA Helicase I Catalyzes a Unidirectional and Highly Processive Unwinding Reaction*

(Received for publication, August 3, 1987)

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DNA helicases catalyze the unwinding of duplex DNA and play an essential role in the metabolism of nucleic acids in the cell. In Escherichia coli at least seven enzymes with helicase activity have been isolated and described (1–9). The reason for the variety of helicases is not understood, but presumably reflects multiple roles for these enzymes in the cell. The E. coli DNA helicases are known to play central roles in DNA replication (10), DNA mismatch repair (11), excision repair (12, 13), and recombination (14). In addition, E. coli helicases are essential for bacteriophage ϕX174 replication (15) and for bacterial conjugation (16).

Helicase I was the first DNA helicase isolated from E. coli (17, 18). This enzyme is a single polypeptide of Mr = 180,000 (17) encoded by the tral gene of the E. coli F factor (19). The F factor, a plasmid of approximately 100 kb,1 is able to transfer its DNA from the host cell (F+) to an F– recipient cell which is in direct physical contact with the host (for a recent review, see Ref. 20). Helicase I is required at the DNA transfer stage of bacterial conjugation (21) and is thought to be involved in unwinding the F plasmid from a site-specific nick at the origin of transfer (19). This unwinding of the F plasmid may provide the single-strand of DNA which is transferred to the recipient bacterium.

Helicase I has been purified and partially characterized biochemically (17, 18). It is a single-stranded (ss) DNA-dependent nuclease 5′-triphosphatase (NTPase) and a helicase. As an NTPase, helicase I is markedly stimulated by a ssDNA cofactor and requires a divalent cation (either magnesium or calcium) for activity (17). The enzyme has been reported to function as a multimer as it (i) readily forms aggregates at low ionic strength, and (ii) shows very low ATPase activity at KCl concentrations above 150 mM, where the enzyme presumably exists in the monomeric state (17, 18, 22). ATP (dATP) appears to be the preferred substrate for the NTP hydrolysis reaction (17). The helicase I unwinding reaction requires concomitant NTP hydrolysis (18, 22, 23). When the unwinding reaction catalyzed by helicase I was measured by S1 nuclease digestion or by velocity sedimentation of the reaction products, helicase I was shown to be capable of unwinding DNA-DNA or RNA-DNA partial duplex structures (18). However, helicase I does not utilize RNA as an NTPase cofactor (17). The mode of action of the helicase appears to be processive (22, 23), and a region of ssDNA adjacent to the duplex DNA of approximately 200 nucleotides in length is necessary for helicase I to initiate an unwinding reaction (2, 23). Helicase I will not unwind completely duplex DNA molecules or nicked DNA molecules (22). Results with exonuclease eroded linear duplex DNA molecules have suggested that helicase I unwinds duplex DNA in the 5′ to 3′ direction with respect to the strand on which the enzyme is bound (23).

In this study we have extended the enzymatic characterization of helicase I both as an ATPase, and as a helicase using an assay which directly measures the unwinding reaction. Helicase I appears to translocate processively along a ssDNA effector using the energy released by NTP hydrolysis to fuel translocation. The enzyme can utilize all eight predominant NTPs as hydrolysis substrates in the helicase reaction. The unwinding of duplex DNA by helicase I is independent of protein concentration with respect to the length of duplex DNA unwound suggesting that the unwinding reaction is processive. Moreover, helicase I translocates extremely slowly from one DNA substrate molecule to another. In addition, we
confirm the direction of unwinding as 5' to 3' with respect to the strand on which the enzyme is bound.

**EXPERIMENTAL PROCEDURES AND RESULTS**

*Purification of Helicase I—*Helicase I was purified as described under "Experimental Procedures"; Table I summarizes the purification. The initial cell extract contained multiple DNA-dependent as well as DNA-independent ATPase activities making it impossible to estimate the total helicase I activity in crude extracts. For this reason, no estimate of overall yield is made. The phosphocellulose column resolves three peaks of DNA-dependent ATPase activity with helicase I eluting in the peak resolved at 250 mM NaCl. All subsequent chromatographic steps yield a single peak of DNA-dependent ATPase activity. The specific activity of helicase I calculated after the hydroxylapatite step of the purification varies from preparation to preparation. This variability is possibly due to an endonuclease which is sometimes present at this stage of the purification. If this endonuclease linearized the DNA substrate used in the ATPase assay the specific activity of helicase I would appear to drop dramatically. The activity of helicase I on linear DNA substrates is discussed in detail below. The final fraction of the helicase I purification contained a single polypeptide that migrated with a M, = 180,000 on a polyacrylamide gel run in the presence of sodium dodecyl sulfate (Fig. 1). Fraction VI contained no detectable endo- or exonuclease activity as determined by lack of detectable degradation of the partial duplex DNA substrates used in the helicase assays.

**Helicase and ssDNA-dependent ATPase Reactions**—The unwinding reaction catalyzed by helicase I was originally characterized using either a coupled assay that measured the fraction of a radioactively labeled DNA substrate rendered susceptible to S1 nuclease, or by velocity sedimentation of the DNA substrate (18, 22, 23). We have extended this characterization using an assay that directly measures the ability of helicase I to unwind a partial duplex DNA molecule (24). This assay has been used to characterize several other DNA helicases (5, 24, 32, 33). The DNA substrate utilized in this assay consists of the complementary strand of a radioactively labeled DNA restriction fragment annealed to circular M13mp7 ssDNA as described under "Experimental Procedures" (see Fig. 4A). The helicase assay measures the fraction of the [32P]DNA fragment displaced by the helicase.

**DNA Substrate Requirements for ATP Hydrolysis**—The helicases characterized to date are all ssDNA-dependent ATPases (1). Table III summarizes the results of experiments performed using several different DNA molecules as effectors of the helicase I-catalyzed ssDNA-dependent ATPase reaction. Circular M13mp7 ssDNA proved to be the best effector of the DNA-dependent ATP hydrolysis reaction. Double-stranded linear (RF III) or supercoiled (RF I) DNA molecules could not substitute for ssDNA. Surprisingly, neither poly(dT) nor linear M13mp7 ssDNA could serve as effectors of the ATP hydrolysis reaction. Since both are ssDNA molecules we expected that they would substitute for circular ssDNA. These results suggest that DNA termini may inhibit the ssDNA-dependent ATPase reaction catalyzed by helicase I. The implications of this result will be discussed later in the text. The concentration of circular ssDNA required to achieve one-half-maximal ATPase reaction velocity (Kcat) was determined. Kcat for circular M13mp7 ssDNA was 0.51 μM.

**The Length of Duplex DNA Unwound Is Independent of Protein Concentration**—To determine the effect of the length of duplex DNA on the unwinding reaction catalyzed by helicase I, three partial duplex DNA substrates were constructed as described under "Experimental Procedures" (Fig. 4A). Since the DNA substrate concentration was essentially the same for each substrate the results obtained with all three are directly comparable. Helicase I displaced greater than 70% of the [32P]DNA fragment from each of the three helicase substrates (Fig. 4B). The fraction of the [32P]DNA fragment displaced from each substrate was directly proportional with enzyme concentration up to approximately 6 ng of helicase I. Interestingly, the same fraction of [32P]DNA fragment was displaced from each helicase substrate at all enzyme concentrations tested. Since the 851-bp partial duplex substrate contains 12-fold more base pairs of duplex DNA than the 71-bp partial duplex substrate, and was unwound to the same extent, the unwinding reaction did not require input of additional protein to unwind longer regions of duplex DNA. Essentially the same results have been obtained using a helicase substrate contain-

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1 Portions of this paper (including "Experimental Procedures," part of "Results," Tables I and II, and Figs. 1-3, and 9) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

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TABLE III

ATP hydrolysis in the presence of DNA

| DNA effector     | Nucleotide concentration (μM) | [3H]ADP formed (pmol) |
|------------------|-------------------------------|-----------------------|
| M13mp7 circular ssDNA | 3.0                            | 654                   |
| M13mp7 linear ssDNA   | 3.4                            | ≤40                   |
| M13mp7 RF I DNA*      | 3.5                            | 54                    |
| M13mp7 RF III DNA*    | 3.3                            | 49                    |
| Poly(dT)             | 3.6                            | 28                    |
| No DNA              | 0.0                            | ≤20                   |

* Supercoiled.

* Duplex linear.
protein concentration was determined by the method of Lowry and co-workers. The reaction mixture was prepared and the reaction stopped by the addition of EDTA and dyes as described under "Experimental Procedures." The data presented represents an average of three or more experiments. C, 71-bp partial duplex circles; O, 343-bp partial duplex circles; C1, 851-bp partial duplex circles.

Fig. 5. Rate of the unwinding reaction. Helicase reactions were as described under "Experimental Procedures" with the following modifications. The reaction mixture volume was increased to 220 μl and 20-μl aliquots were removed for heat-denatured substrate and no helicase I controls. Helicase I (10.5 ng) was added to the remaining 180 μl and the reaction was placed at 37 °C. Aliquots (20 μl) were removed at the indicated times and the reaction stopped by the addition of EDTA and dyes as described under "Experimental Procedures." The data presented represents an average of three or more experiments. C, 71-bp partial duplex circles; O, 343-bp partial duplex circles; C1, 851-bp partial duplex circles.

the reaction conditions employed, the unwinding reaction was essentially complete in 10 min at 37 °C. The kinetics of the unwinding reaction were very similar for all three DNA substrates (Fig. 5). The rate of the unwinding reaction was linear for the first 6 min, then decreased until the rate of unwinding was essentially zero after 15 min. Therefore, at early reaction times (less than 6 min), approximately 12-fold more base pairs were unwound on the 851-bp partial duplex substrate than on the 71-bp partial duplex substrate.

Since helicase I does not appear to turn over from one circular DNA substrate molecule to another, we investigated the kinetics of the unwinding reaction using a linear helicase substrate (Fig. 7). On a linear DNA substrate the enzyme should encounter an end of the DNA molecule and may be forced to dissociate and seek a new DNA substrate. This may contrast with what occurs on a circular DNA molecule where the enzyme may be able to translocate indefinitely.

Linear helicase substrates were produced by taking advantage of the single ClaI restriction site in the duplex region of the circular 343-bp partial duplex substrate. Cleavage with this enzyme will generate a linear substrate with 141 bp of unlabeled duplex DNA at the 5' end of the molecule and 202 bp of [32P]DNA duplex at the 3' end of the molecule separated by 6895 nucleotides of ssDNA. As helicase I unwinds duplex DNA in a 5' to 3' direction, the [32P]DNA fragment will be displaced from the 3' end of the linear substrate. When kinetic experiments were carried out using this linear substrate (202 bp linear) there was no apparent increase in helicase I turnover (Fig. 7); the kinetics were the same as those observed using a 71-bp partial duplex circular DNA substrate.

To ensure that the presence of the duplex DNA ends was not inhibiting the enzyme, the same studies were carried out using linear DNA substrates with the duplex region located internally. These substrates were constructed by taking advantage of the short duplex hairpin loop created in the ssDNA by the polylinker region of M13mp7. The BamHI restriction endonuclease site present in the hairpin was used to produce linear helicase substrates. Complete digestion at the BamHI site produced a linear partial duplex DNA substrate from a circular substrate. The kinetics of unwinding on this 71-bp linear substrate were also identical to the unwinding kinetics observed using the 71-bp partial duplex DNA substrate (Fig. 7). However, the extent of the unwinding reaction on linear substrates can be lower than on circular helicase substrates depending on where the duplex DNA is located relative to the ssDNA ends. Assuming that helicase I translocates 5' to 3' along ssDNA, a fraction of the helicase I molecules will bind to ssDNA and never encounter a region of duplex DNA on the 3' side of the binding site. This results in apparent lower
unwinding activity by helicase I on certain linear substrates (data not shown). The above data suggest that helicase I does not rapidly dissociate from the end of a linear DNA molecule and bind to a new DNA molecule. In fact, helicase I may remain bound at the end of the linear DNA molecule.

**Kinetics of the ssDNA-dependent ATPase Reaction**—ATP hydrolysis was required for the unwinding reaction (see Table II) and it seems reasonable to assume that the two activities may be coupled. For this reason we investigated the kinetics of the ssDNA-dependent ATPase reaction catalyzed by helicase I using both circular and linear DNA effectors. After a brief lag phase the rate of the ATP hydrolysis reaction was linear with time for more than 30 min using either circular M13mp7 ssDNA (Fig. 8A) or the circular 851-bp partial duplex helicase substrate (Fig. 8B) as a DNA effector. This result offers a sharp contrast to what was observed when the unwinding reaction was monitored (see Fig. 5). The rate of the unwinding reaction leveled off to essentially zero after approximately 15 min. Clearly the enzyme continued to hydrolyze ATP after the unwinding reaction had ceased.

When linear M13mp7 ssDNA or the linear 851-bp helicase substrate were used as DNA effectors of the ssDNA-dependent ATPase activity, very little ATP hydrolysis was measured (Fig. 8, A and B). When the kinetics of the ATPase reaction using a linear DNA effector was compared to the kinetics of the unwinding reaction on the same linear molecule, an interesting contrast was seen. ATP hydrolysis in the presence of a linear DNA effector was negligible compared to ATP hydrolysis in the presence of a circular DNA effector. However, helicase activity on linear and circular DNA substrates was equivalent (see Fig. 7). One explanation for these results assumes that helicase I translocates unidirectionally in the 5' to 3' direction to the end of a linear molecule and stops, no longer requiring ATP hydrolysis for translocation or unwinding. On circular DNA substrates the enzyme may be able to translocate indefinitely resulting in a linear ATPase reaction.

The role of ATP hydrolysis in the helicase reaction is not clear. However, it is likely that the energy released by hydrolysis of ATP is utilized by helicase I for processive translocation along ssDNA and perhaps for unwinding the DNA duplex. A kinetic parameter, $K_{eff}$, has been utilized to define helicase reaction mechanisms on ssDNA. $K_{eff}$ is defined as the amount of ssDNA required to achieve one-half the maximal rate of ATP hydrolysis. If the $K_{eff}$ is substantially greater for linear DNA molecules than for circular DNA molecules, this can be interpreted as evidence for a processive translocation mechanism (24, 27).

To examine whether helicase I exhibits a processive translocation mechanism on ssDNA, $K_{eff}$ values were determined for both circular and linear M13mp7 ssDNA. The $K_{eff}$ value for M13mp7 circular ssDNA is 0.5 μM DNA (Fig. 9); the value for a linear DNA molecule cannot be determined as the rate of ATP hydrolysis on a linear DNA effector is at or below detectable limits under the conditions used. However, the results of unwinding assays using the linear helicase substrate indicated that the enzyme did indeed translocate over the linear DNA molecule. This suggests that helicase I is extremely processive, dissociating very infrequently from ssDNA. In fact, the enzyme apparently remains bound to the end of a linear DNA molecule even when the enzyme is not hydrolyzing ATP.

**FIG. 6. Extent of the unwinding reaction at different helicase I concentrations.** Panel A, kinetic analysis of the helicase I unwinding reaction using the 348-bp partial duplex substrate. Helicase reactions were as described under "Experimental Procedures" with the following modifications. The reaction volume was increased to 220 μl and 20-μl aliquots were removed for the heat denatured and no helicase I controls. O, 10.5 ng or ★, 27 ng helicase I was added to the remaining 180 μl and the reaction was placed at 37°C. Aliquots (20 μl) were removed at the indicated times and the reaction was stopped by the addition of EDTA and dyes as described under "Experimental Procedures." Panel B, kinetic analysis of the helicase I unwinding reaction using the 851-bp partial duplex substrate. Helicase reactions were as described under "Experimental Procedures" with the following modifications. The reaction volume was increased to 300 μl and 20-μl aliquots were removed for the heat denatured and no helicase I controls. Helicase I (31 ng) was added to the remaining 260 μl and the reaction was placed at 37°C. Aliquots (20 μl) were removed at the indicated times (★). At 15 min a parallel reaction was started by taking a portion (130 μl) of the reaction mixture and adding an additional 31 ng of helicase I to this aliquot. This second reaction mixture continued to incubate at 37°C and 20-μl aliquots were removed at the indicated times (O). The data presented here represents an average of two experiments.
which contained a substrate. This indicated a need for ATP hydrolysis concomitantly with unwinding of duplex DNA. All eight of the commonly occurring predominant NTPs (dNTPs) were effectively utilized by helicase I as hydrolysis substrates in place of ATP in the unwinding reaction.

Helicase I requires a region of ssDNA for binding of the enzyme and does not unwind a fully duplex molecule (22). In fact, the ssDNA must be of a specific polarity in relation to the duplex DNA in order for an unwinding reaction to occur. This is consistent with the fact that all helicases known to date unwind duplex DNA with a specific directionality. The direction of the unwinding reaction catalyzed by helicase I is 5' to 3' with respect to the strand on which the enzyme is bound. This was demonstrated using a linear ssDNA molecule with duplex ends (see Fig. 3A). Thus helicase I unwinds duplex DNA in the same direction as the E. coli DnaB protein (5) and helicase III (1) and in the opposite direction of Rep protein (3), helicase II (28), and the E. coli 75-kDa helicase (7).

The unwinding reaction catalyzed by helicase I was independent of protein concentration with respect to the length of the duplex region unwound. This was demonstrated using three partial duplex substrates with duplex regions ranging from 71 to 851 bp in length. A specified concentration of helicase I displaced the same fraction of $[^{32}P]DNA$ fragment from each partial duplex substrate. Thus the fraction of DNA substrate molecules unwound by helicase I is independent of the length of the duplex region on the substrate. In fact, a duplex region of 2.5 kb in length could be unwound to a comparable extent using the same concentration of helicase I. Since no additional protein was required to unwind longer duplex DNA regions the mechanism of the unwinding reaction appears to be processive.

Consistent with a processive unwinding mechanism is the apparent slow turnover of helicase I molecules from one DNA substrate to another. The extent of the reaction, as defined by the fraction of substrate unwound when the plateau was reached, was directly proportional with helicase I concentration on both circular and linear partial duplex DNA substrates. Since the enzyme remained active for more than 40 min and the DNA substrate was competent to be further unwound, we interpret this result as indicating that helicase I does not turn over to a new DNA substrate at any significant rate. The results were the same even when a DNA terminus was provided, as on the linear partial duplex molecules.

The kinetics of the ssDNA-dependent ATP hydrolysis reaction were quite different on linear and circular DNA effectors (see Fig. 8, A and B). The ATP hydrolysis reaction was linear with time for more than 40 min on a circular DNA molecule; ATP hydrolysis was barely detectable on a linear DNA molecule. This was true of both ssDNA effectors and partial duplex DNA helicase substrates used as effectors. Since ATP hydrolysis is required for unwinding of duplex DNA and helicase I has been shown to unwind a duplex region on a linear molecule, it seems reasonable to conclude that ATP hydrolysis fuels the 5' to 3' translocation of helicase I along ssDNA as well as movement through duplex DNA.

A comparison of the results of ATP hydrolysis assays and unwinding assays on both linear and circular DNA molecules presents an interesting contrast. A linear partial duplex DNA substrate was fully functional as a helicase substrate but did not appear to be a functional effector for ATP hydrolysis. Circular DNA molecules, on the other hand, provide good helicase substrates and were effectors of the ATP hydrolysis reaction. This conflict can be explained if helicase I remains bound on a circular DNA molecule for an indefinite period of time and continues to translocate and hydrolyze ATP. When
a duplex region of DNA is encountered, the duplex is unwound but the enzyme remains bound to the ssDNA and does not turn over to a new DNA substrate molecule. On a linear DNA molecule the enzyme migrates in the 5' to 3' direction utilizing ATP hydrolysis to fuel translocation, but stops when an end is reached. At this point ATP hydrolysis also ceases. If duplex DNA is encountered during the 5' to 3' migration an unwinding reaction takes place. The enzyme subsequently dissociates from the end of the linear DNA molecule or associates with a new DNA molecule very slowly. Alternatively the active enzyme species could be a multimer which must dissociate to form monomers prior to binding a new substrate molecule. The result of this slow step is that ATP hydrolysis is barely detectable when a linear DNA molecule is used as an effector of ATP hydrolysis. Interestingly, this provides an explanation for why linear homopolymer DNA molecules were not effective in stimulating the ATP hydrolysis reaction catalyzed by helicase I (17). In addition, this also suggests that only a low level of ATP hydrolysis is required to fuel translocation along ssDNA and for unwinding regions of duplex DNA. Whether the energy released in hydrolyzing ATP is utilized solely in reaching duplex DNA or is also required for the separation of duplex DNA strands is not clear at present. However, it should be noted that no substantial increase in ATP hydrolysis was observed when linear partial duplex substrates were used as effectors of the ATP hydrolysis reaction as compared to linear ssDNA (see Fig. 8).

The biochemical properties of helicase I are suitable for the role it is thought to play in bacterial conjugation. The enzyme is a highly processive helicase capable of unwinding long regions of duplex DNA. However, the purified enzyme will not initiate an unwinding reaction on a nicked DNA molecule (22). Many DNA helicases have shown a dependence on, or interactions with other proteins in order to provide optimal helicase activity (3, 5, 9, 14). As knowledge of the enzymology of bacterial conjugation increases it will be interesting to see whether a protein involved in the replication or transfer of the F factor will be required to aid in the functioning of helicase I. Perhaps an enzyme will be found which enables helicase I to unwind the F factor from the strand-specific nick known to occur at the origin of transfer.

Acknowledgments—We would like to thank Dr. Timothy Lohman, Edgar Wood, James George, and Dr. Robert Lahue for critical reading of this manuscript. In addition, we would like to thank Susan Whitfield for the preparation of the artwork.

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Fig. 8. ATP hydrolysis in the presence of ssDNA effectors. ATP hydrolysis was measured as described under "Experimental Procedures." Panel A, ATP hydrolysis versus time using M13mp7 ssDNA as an effector. The volume of the reaction mixture was increased to 40 µl and a 5-µl aliquot was removed for a no helicase I control. Helicase I (21 ng) was added to the remainder of the reaction mixture and the reaction was placed at 37 °C. Aliquots (5 µl) were removed at the indicated times and the reaction stopped as described under "Experimental Procedures." ○, M13mp7 ssDNA linearized with BamHI, ●, M13mp7 ssDNA circles. Panel B, ATP hydrolysis versus time using the 851-bp partial duplex substrate as an effector. The volume of the reaction mixture was increased to 60 µl and a 5-µl aliquot was removed for a no helicase I control. Helicase I (3.6 ng) was added to the remainder of the reaction mixture and the reaction was placed at 37 °C. Aliquots (5 µl) were removed at the indicated times and the reaction stopped as described under "Experimental Procedures." ○, 851-bp partial duplex substrate linearized with BamHI as described under "Results"; ●, circular 851-bp partial duplex substrate. The 851-bp partial duplex substrate concentration in the reaction mixtures was approximately 1.5 µM for both linear and circular substrates. Data presented is an average of two or more experiments. Background values have been subtracted for all data presented.
SUPPLEMENTARY MATERIAL TO
SUPPLEMENTARY DNA HELICASE I CATALYZES A UNIDIRECTIONAL AND SHORT PROGRESSIVE \nENWINDING REACTION

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EXPERIMENTAL PROCEDURES

Materials

Buffers - Helicase I was purified as described below. Restriction endonucleases were purchased from either Bethesda Research or New England Biolabs. Reaction conditions were chosen suggested by the manufacturer. 32P-labeled DNA (large fragment) was purchased from ICN Biochemica. Luciferase was purchased from Sigma Chemicals. Helicase II was purified as described (24) and was the kind gift of J.W. Woolsey (Univ. of North Carolina).

UTP and 32P-UTP - Page H1252a 32P-UTP and radioactive form of I N HTP were prepared as described (33). All unlabeled nucleotides were purchased from P-L Biochemicals. 32P-UTP was purchased from ICN Biochemica. [3-3H]UTP was obtained from New England Nuclear.

Methods

Purification of helicase I - Helicase I was purified by modification of the procedure of Abdel-Monem and Hoffmann-Berling (17). The lanes of a 0.7% (w/v) 17.5 cm agarose gel were loaded with an equal volume of a sonicated control (10 µg DNA/ml) and a 10 µg/ml DNA sample. The gel was run at 70 V for 2 hr.

DNAase I digestion - DNA was isolated from E. coli by the method of Grossman and其中包括的分子间相互作用

Fraction 4 was loaded onto a phosphocellulose column (5.5 cm x 5.5 cm) equilibrated with buffer A and eluted with 0.5 M NaCl in buffer A. Fractions were assayed for dependent DNA activity described below by running samples on 10% polyacrylamide gels in the absence of DNA. The DNA-dependent ATPase activity eluted at 0.25 M NaCl in buffer A. Fractions 3 and 4 were pooled and dialyzed overnight against buffer A (160 ml). Any precipitate in the dialysate was removed by centrifugation at 37,000 x g for one hour. The supernatant solution (Fraction 1) was concentrated to 100 µl by dilution with buffer A.

Fraction 1 was loaded onto a phosophocelulose column (5.5 cm x 5.5 cm) equilibrated with buffer A and eluted with 0.2 M NaCl in buffer A. Fractions were assayed for dependent DNA activity as described below by running samples on 10% polyacrylamide gels in the absence of DNA. The DNA-dependent ATPase activity eluted at 0.25 M NaCl in buffer A. Fractions 3 and 4 were pooled and dialyzed overnight against buffer A (160 ml). Any precipitate in the dialysate was removed by centrifugation at 37,000 x g for one hour. The supernatant solution (Fraction 1) was concentrated to 100 µl by dilution with buffer A.
E. coli DNA Helicase I

McgC1 concentrations up to 8 mM (data not shown). The helicase reaction also required the presence of a hydrolyzable NTP. None of the nonhydrolyzable ATP analogs tested were capable of substituting for ATP in the unwinding reaction (Table I), suggesting that the helicase reaction requires concomitant ATP hydrolysis. The helicase reaction was also inhibited by NaiC1 concentrations as high as 75 mM.

**TABLE I**

| Reaction Component | [32P]DNA Fragment (nM) | [32P]ADP formed (nM) |
|--------------------|------------------------|----------------------|
| Complete           | 50                     | 0.23                 |
| -NaiC1             |                         |                      |
| -NaiC1, +4M EDTA    | C3                     | nd                   |
| -ATP               | C3                     | nd                   |
| -ATP, +TTF[32P]     | C3                     | nd                   |
| -ATP, +2M γ-ATP     | C3                     | nd                   |
| -ATP, +APDγBP      | C3                     | nd                   |
| FVIIIb S1C1         | C3                     | 0.02                 |

Helicase activity was measured in the standard helicase assay, with the indicated modifications, as described under "Experimental Procedures" using the 343 bp partial duplex substrate.

**Fig. 1.** DNA-polyuridylates gel electrophoretogram of purified helicase I. Comassie blue stained 12% polyacrylamide gel. Lane 1, molecular weight standards: ovalbumin, 42.7 kDa; bovine serum albumin, 66 kDa; phosphorylase B, 97.4 kDa; β-galactosidase, 116 kDa. Lane 2, protein: 200 kDa. Lane 3, 5 g of purified helicase I (Fraction V3).

**Fig. 2.** Helicase I utilizes all NTP's in the unwinding reaction. Helicase reactions were as described under "Experimental Procedures" using 2.5 ng lanes 1, 7, 9, 11, 15, 17, 19, 21 or 2.2 ng lanes 2, 4, 8, 12, 14, 16, 18, 20) of lanes I and I of the partial duplex DNA substrate. Lane 1 was the heat denatured 343 bp substrate. Lane 3 was the no helicase control. Lane 5 was a control containing 1.0 μM CTP, 1.5 μM ATP, 1.0 μM GTP, 1.5 μM UTP, 1.5 μM GTP, 1.0 μM UTP, and 1.5 μM ATP.

**Fig. 3.** Helicase I unwinds DNA in a 5' to 3' direction. Panel A: Linear partial duplex DNA substrate used to determine the direction of the unwinding reaction, asterisks denote the position of the radiolabeled label. Panel B: Helicase reactions were as described under "Experimental Procedures" Lane 1, heat denatured direction substrate shows in panel A; lane 2, heat denatured 343 bp substrate, lane 3, helicase I and the 343 bp substrate; lane 4, 343 bp substrate and 75 μg helicase I; lane 5, direction substrate and 343 bp substrate; lane 6, no helicase I and the 343 bp substrate and 17 mg helicase I; lane 7, direction substrate and 17 mg helicase I; lane 8, direction substrate and 17 mg helicase I.

**Fig. 9.** Helicase I is highly processive. ATP hydrolysis was measured as described under "Experimental Procedures". Lane 1, gel mask for initial ATP concentration. The gel mask was used to determine the DNA effector concentration. Lines were determined by linear regression analysis.