Escherichia coli DNA helicase II (uvrD gene product) catalyzes the unwinding of DNA-RNA hybrids in vitro

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Communicated by Charles C. Richardson, March 27, 1989 (received for review February 3, 1989)

ABSTRACT DNA helicase II is a well-characterized Escherichia coli enzyme capable of unwinding duplex DNA and known to be involved in both methyl-directed mismatch repair and excision repair of pyrimidine dimers. Here it is shown that this enzyme also catalyzes the ATP-dependent unwinding of a DNA-RNA hybrid consisting of a radioactively labeled RNA molecule annealed on M13 single-stranded DNA. The DNA-RNA unwinding reaction required less protein to unwind more base pairs than the corresponding unwinding of duplex DNA. In addition, the rate of unwinding of the DNA-RNA hybrid was more than an order of magnitude faster than unwinding of a DNA partial duplex of similar length. The unwinding of the DNA-RNA hybrid is a property unique to helicase II since helicase I, Rep protein, and helicase IV failed to catalyze the reaction. In light of these results it seems likely that helicase II is involved in some previously unrecognized aspect of nucleic acid metabolism, in addition to its known roles in DNA repair reactions.

Escherichia coli cells contain at least eight DNA helicases, enzymes that catalyze the unwinding of duplex DNA in a reaction that depends on the concomitant hydrolysis of a nucleoside 5'-triphosphate (1, 2). Each of these enzymes is apparently involved in a different aspect of DNA metabolism, which may account for the large number of enzymes able to catalyze the same basic reaction. In addition, the E. coli termination factor Rho has been shown to be capable of catalyzing the ATP-dependent unwinding of an RNA-DNA hybrid (3). This activity of Rho protein presumably facilitates the release of RNA transcripts from the DNA template at Rho-dependent terminators.

Considerably less is known about the helicase enzymes present in eukaryotic cells. However, a number of enzymes capable of unwinding duplex DNA have been identified in several cell types (4–8). In addition, enzymes able to catalyze the unwinding of duplex RNA have been detected in eukaryotic cells (9–11). It is likely that these enzymes are involved in various aspects of the biogenesis and interactions of RNA molecules within the cell. The eukaryotic translation initiation factor eIF-4A has also been shown to have an intrinsic RNA-unwinding activity, which is apparently important in removing RNA secondary structure as the mRNA is scanned for initiation codons (12). Thus, helicases capable of unwinding various nucleic acid duplexes have been reported in both prokaryotes and eukaryotes. These enzymes participate in myriad biological processes fundamental to the metabolism of nucleic acids in the cell.

E. coli DNA helicase II was originally described as a duplex DNA-unwinding enzyme (13, 14). This protein has since been shown to be directly involved in methyl-directed mismatch repair (15) and in the excision repair of damage induced in DNA by ultraviolet radiation (16, 17). In addition, genetic experiments have suggested possible roles in recombinination (18), transposition (19), and DNA replication (20). When assayed in vitro, helicase II requires a region of single-stranded DNA (ssDNA) on which to bind and initiate translocation (13, 14, 21), and the unwinding occurs in a 3' → 5' direction with respect to the bound ssDNA (22). The helicase reaction requires stoichiometric amounts of protein (13, 14, 21), and concomitant ATP hydrolysis is necessary for unwinding to be observed. These biochemical characteristics of the unwinding reaction are consistent with the known roles for helicase II in unwinding relatively short regions of duplex DNA.

Recently both RNA-RNA and RNA-DNA helicases have been shown to contain sequence similarity with several prokaryotic DNA helicases, including helicase II (23). For this reason it was of interest to determine whether or not these prokaryotic helicases could unwind DNA-RNA hybrids. Such an activity could be important in several aspects of nucleic acid metabolism. Thus the ability of four E. coli helicases (helicase I, helicase II, helicase IV, and Rep protein) to catalyze the unwinding of a DNA-RNA hybrid was examined. In this report I describe the efficient and rapid unwinding of a DNA-RNA hybrid catalyzed by helicase II, an enzyme previously thought to unwind only duplex DNA.

MATERIALS AND METHODS

Enzymes. Bacteriophage T7 RNA polymerase and E. coli DNA polymerase I (large fragment) were from United States Biochemical. Helicase II (21), helicase I (24), Rep protein (25), and helicase IV (1) were purified as previously described.

DNA and Nucleotides. M13 phage were grown in E. coli 71.18 (26) and ssDNA was purified as described (27). M13mp7-917 is a derivative of M13mp7 that contains an 1100-base-pair (bp) insert (Pvu II–EcoRI) from the 5' end of the uvrD gene (28) cloned into the EcoRI site in the M13mp7 polynucleotide. pBSRI-917 is a derivative of the Bluescript plasmid (Stratagene) containing the same insert cloned into the EcoRI site in the plasmid polynucleotide. Unlabeled nucleotides were from Pharmacia P-L Biochemicals. [α-32P]UTP and [α-32P]dCTP were from Amersham.

DNA-RNA Hybrid Substrate Construction. Transcription reaction mixtures (100 μl) designed to produce the [32P]RNA transcript contained 40 mM Tris/HCl buffer (pH 7.5); 10 mM MgCl2; 5 mM dithiothreitol; 500 μg of bovine serum albumin per ml; 500 μM each ATP, CTP, and GTP; 50 μM unlabeled UTP, 200 μCi of [α-32P]UTP (μCi = 37 kBq), 12 units of T7 RNA polymerase, and ~1 μg of DNA template. The DNA template was pBSRI-917 cleaved with Bgl II, which yields three fragments, one of which contains the T7 RNA polymerase promoter and a portion of the insert. This fragment was purified by polyacrylamide gel electrophoresis and electrophoresis was used directly as the template in run-off transcription reactions producing the labeled RNA molecule. Transcription mixtures were incubated for 30 min at 37°C.

Abbreviations: ssDNA, single-stranded DNA; nt, nucleotid(s).
RQI DNase (1 unit, Promega) was added and incubation was continued for an additional 15 min at 37°C. The reaction mixture was extracted with an equal volume of phenol and labeled RNA was purified by gel filtration through a Sepharose CL-6B column. The elution buffer contained 10 mM Tris/HCl (pH 8.0), 1 mM EDTA, and 100 mM NaCl. Fractions were analyzed by electrophoresis through a polyacrylamide gel under denaturing conditions, and those containing the specific transcript were pooled and used directly in annealing reactions. Annealing of the \(^{32}P\)RNA transcript to M13mp7-917 ssDNA was accomplished by mixing appropriate amounts of RNA and ssDNA in a reaction mixture with 60 mM Tris/HCl (pH 7.5), 10 mM MgCl\(_2\), 5 mM diethio-
tol, and 50–100 mM NaCl and heating at 95°C for 3 min followed by incubation at 65°C for 1 hr. The resulting DNA-RNA hybrid was used directly in unwinding reactions.

The DNA partial duplex substrate was constructed as follows. The Bluescript derivative pBSRI-917 was cleaved with Sac II, which yields two fragments, one of which hybridizes with M13mp7-917 ssDNA. This fragment was purified by polyacrylamide gel electrophoresis and electro-

cution and hybridized with M13mp7-917 ssDNA as described above. The resulting duplex substrate was labeled at its 3' terminus by using \(\alpha\)\(^{32}P\)dCTP (21). The labeled partial duplex DNA substrate was purified on a Bio-Gel A-5m (Bio-Rad) column and used directly in helicase assays.

**Helicase Assays.** The standard unwinding reaction mixture (20 µl) contained 40 mM Tris/HCl (pH 7.5), 4 mM MgCl\(_2\), 5 mM dithiothreitol, 50 µg of bovine serum albumin per ml, 2 mM ATP, 5 mM NaCl, 2.5% (vol/vol) glycerol (contributed by the enzyme preparation), 3 units of RNasin (Promega), 5.5 µM DNA substrate (nucleotide equivalents), and the indicated amount of helicase II. Reactions were incubated at 37°C for 10 min unless otherwise indicated. Reactions were terminated by the addition of 10 µl of 40% glycerol/30 mM EDTA/0.1% xylene cyanol/0.1% bromophenol blue and the reaction products were resolved by electrophoresis in a 6% polyacrylamide gel (preelectrophoresed at 350 V for 1 hr) containing 7 M urea at 350 V for 1–2 hr. Polyacrylamide gels were analyzed by film autoradiography, and the labeled products were quantitated by cutting the gel into 1-cm slices and measuring the radioactivity with a liquid scintillation counter. All solutions were prepared using H\(_2\)O treated with diethyl pyrocarbonate (29).

**RESULTS**

The substrate used to measure the ability of helicase II to catalyze the unwinding of a DNA-RNA hybrid is shown in Fig. 1A. A 313-nt \(^{32}P\)RNA molecule was annealed onto an M13mp7 ssDNA derivative that contained a region of DNA complementary to 244 nt of the RNA molecule. The resulting DNA-RNA hybrid contained 244 bp of DNA-RNA duplex and a 69-nt 5'-single-stranded RNA tail.

The unwinding of the DNA-RNA hybrid was detected by resolving the products of the reaction in a 6% polyacrylamide gel. The requirements of the DNA-RNA unwinding reaction are shown in Fig. 1B. The helicase reaction required both ATP and MgCl\(_2\). Substitution of a nonhydrolyzable ATP analogue, adenosine 5'-(β,γ-imido)triphosphate, for ATP did not support the unwinding reaction, indicating that ATP hydrolysis was required. dATP could be substituted for ATP as the nucleotide cofactor with similar results and at similar concentrations (data not shown). The DNA-RNA unwinding reaction was resistant to increasing ionic strength (Fig. 1B, lanes 7–10). In fact, NaCl concentrations up to 200 mM did not inhibit the unwinding of the DNA-RNA helix (data not shown).

![Fig. 1](image1.png)

**Fig. 1.** Substrate and reaction conditions for helicase II unwinding of a DNA-RNA hybrid. (A) The DNA-RNA hybrid substrate consists of a 313-nucleotide (nt) \(^{32}P\)RNA molecule annealed to a derivative of M13mp7 ssDNA. The duplex region is 244 bp long. (B) Unwinding reaction mixtures were as described under Materials and Methods with the indicated modifications; each (except lanes 1 and 2) contained 38.5 ng of helicase II (Hel I). Lane 1, no-enzyme control; lane 2, heat-denatured (3 min at 100°C) substrate control. Adenosine 5'-[β,γ-imido]triphosphate (AMP-PNP) was substituted for ATP at a concentration of 2 mM. EDTA was included at a final concentration of 10 mM. The fraction of the \(^{32}P\)RNA fragment displaced in lane 3 was 50%.

Biochemical and genetic data indicate that DNA helicase II is involved in at least two DNA repair pathways (15–17), presumably as a DNA-unwinding enzyme. Thus, it was of interest to directly compare the unwinding of duplex DNA with the unwinding of the DNA-RNA hybrid. The partial duplex DNA substrate constructed for this purpose consisted of 243 bp of duplex DNA with a 50-nt ssDNA 5' tail. This substrate is essentially identical with the DNA-RNA hybrid in terms of secondary structure. Moreover, the same nucleotide sequences are base-paired in each of the substrates.

A direct comparison of helicase II unwinding of the DNA duplex with unwinding of the DNA-RNA hybrid is shown in Fig. 2. Both unwinding reactions were relatively linear with increasing protein concentration. However, at all helicase II

![Fig. 2](image2.png)

**Fig. 2.** Helicase II unwinds a DNA-RNA hybrid more efficiently than does duplex DNA. Standard reaction mixtures contained either the DNA-RNA hybrid (○) or the DNA partial duplex (○) substrate at a concentration of 5.5 µM (nucleotide equivalents), 80 mM NaCl, and the indicated amount of helicase II. The percentage of labeled fragment displaced was calculated as described (30). The data represent the average of three or more separate experiments (individual values were within 1 SD of the mean).
concentrations tested, substantially more of the DNA-RNA hybrid was unwound as compared to the duplex DNA substrate. With 40 ng of helicase II, >50% of the RNA fragment was unwound from the circular DNA molecule, whereas <7% of the DNA fragment was unwound. In this case, helicase II unwound 1.4 × 10^{12} bp of DNA-RNA hybrid while unwinding only 1.5 × 10^{11} bp of duplex DNA. Similar differences in the unwinding of the DNA duplex as compared to the DNA-RNA hybrid were observed at all concentrations of helicase II tested. Thus the unwinding of the DNA-RNA hybrid is substantially more efficient than unwinding of the DNA duplex in terms of the amount of helicase II required to unwind a given number of base pairs.

The rates of the two unwinding reactions were also compared using the same two substrates (Fig. 3). At the concentration of helicase II tested (compare with 77 ng in Fig. 2), the unwinding of the DNA-RNA hybrid was rapid, with >70% of the [32P]RNA fragment unwound from the circular DNA in the first 4 min of the reaction. After 4 min the rate of unwinding decreased and ultimately reached zero with somewhat more than 80% of the hybrid substrate unwound. Only rarely was 100% unwinding of the DNA-RNA hybrid observed. This is likely due to the fact that helicase II translocates processively along ssDNA (21) and dissociates to bind a new substrate molecule infrequently.

Unwinding of the DNA partial duplex was relatively linear over the entire 32-min incubation and considerably slower than unwinding of the DNA-RNA hybrid. The rate at which the DNA-RNA hybrid was unwound was more than an order of magnitude higher than the rate at which the DNA duplex substrate was unwound. Assuming that 100% of the enzyme was active (there were ~60 helicase II molecules per DNA substrate molecule in these experiments), the rate at which the DNA-RNA hybrid was unwound (measured at 1 min) was

![Graph](image)

**Fig. 3.** Kinetic analysis of the unwinding of the DNA-RNA hybrid compared to unwinding of a DNA partial duplex. Reactions were as described under Materials and Methods with the following modifications. The reaction mixture contained 80 mM NaCl and the volume was increased to 100 μl. The mixture was assayed on ice without enzyme, and two 20-μl aliquots were removed for the no-enzyme and heat-denatured substrate controls. The remaining 120 μl was incubated at 37°C for 5 min prior to the addition of 462 ng of helicase II. Aliquots (20 μl) were removed at the indicated times and added directly to glycerol/EDTA/dyes for electrophoresis. Reaction products were resolved in a 6% polyacrylamide gel and quantitated by linear scintillation counting of gel slices. The data presented represent the average of three or more experiments (individual values were within 1 SD of the mean). ○, DNA-RNA hybrid substrate; ●, partial duplex DNA substrate.

![Image](image)

**Fig. 4.** Unwinding of a DNA-RNA hybrid by helicase I, helicase II, helicase IV, and Rep protein. Reaction mixtures contained 80 mM NaCl. Lane 1, heat-denatured control; lane 2, no-enzyme control; lane 3, 38.5 ng of helicase II; lane 4, 77 ng of helicase II; lane 5, 5 ng of helicase I; lane 6, 10 ng of helicase I; lane 7, 5 ng of helicase IV; lane 8, 10 ng of helicase IV; lane 9, 25 ng of Rep protein; lane 10, 50 ng of Rep protein. The amounts of helicase I, helicase IV, helicase II, and Rep protein utilized in this experiment were sufficient to unwind 40–80% of a 71-bp partial duplex substrate at a substrate concentration of 2 μM (data not shown).

2 bp per minute per enzyme molecule. The rate of unwinding of the DNA partial duplex was 0.1 bp per minute per enzyme molecule. This rate of unwinding of the DNA partial duplex compares favorably with data reported previously (21). Lower concentrations of helicase II yield a similar rate for unwinding of the DNA-RNA hybrid (data not shown).

To show that the DNA-RNA unwinding reaction was a biochemical characteristic unique to helicase II, the abilities of four *E. coli* helicases to unwind the DNA-RNA hybrid were compared (Fig. 4). Helicase II, Rep protein, and helicase IV all unwind duplex DNA in the 3′ → 5′ direction (refs. 1, 22, and 31; unpublished results). DNA helicase I unwinds duplex DNA in the 5′ → 3′ direction (24). Of the four helicase enzymes tested, only helicase II catalyzed substantial unwinding of the DNA-RNA hybrid. Helicase IV catalyzed some unwinding of the DNA-RNA hybrid, albeit at least an order of magnitude less than the unwinding catalyzed by helicase II. Helicase I also catalyzed slight unwinding of the DNA-RNA hybrid; such a reaction was reported previously (32). The extensive unwinding of a DNA-RNA hybrid does not appear to be a common property of the *E. coli* helicase enzymes; rather it is a specific property of helicase II.

**DISCUSSION**

In light of the data presented above, I suggest that helicase II may be involved in some aspect of RNA metabolism in the cell in addition to its known roles in excision repair (16, 17) and methyl-directed mismatch repair (15). The unwinding of the DNA-RNA hybrid catalyzed by helicase II displayed several characteristics that suggest that it has physiological relevance. (i) The unwinding reaction was substantially more efficient than unwinding of duplex DNA. At all concentrations of helicase II tested, nearly 10-fold more base pairs of the hybrid substrate were unwound as compared to unwinding of the duplex DNA substrate. (ii) The rate of unwinding of the DNA-RNA hybrid was more than an order of magnitude faster than the rate of unwinding of the DNA partial duplex. (iii) The DNA-RNA hybrid unwinding reaction depends on concomitant ATP hydrolysis, as does the DNA unwinding reaction. (iv) The DNA-RNA unwinding reaction was resistant to increasing ionic strength in the physiological range. (v) Helicase II was the only enzyme of the four *E. coli* enzymes tested that catalyzed substantial unwinding of the DNA-RNA hybrid substrate, suggesting that this is not a property common to many helicase enzymes.

That lower amounts of helicase II unwind more of the DNA-RNA hybrid at a higher rate than unwinding of duplex
DNA is of particular interest. Helicase II is known to interact with ssDNA but appears not to interact with single-stranded RNA (13, 14, 21). In the process of unwinding duplex DNA, helicase II may interact with both strands of the duplex. If the interaction with one strand is antagonistic to unidirectional movement along the other strand this may result in lower rates of unwinding. Helicase II might compensate for this antagonistic interaction by releasing stoichiometric amounts of enzyme to unwind duplex DNA. This notion is consistent with the observation of a protein concentration-dependent unwinding of duplex DNA (21). However, since helicase II does not interact with RNA, the DNA-RNA hybrid unwinding reaction could occur by a more processive mechanism and at a higher rate.

What role (if any) might the helicase II unwinding of a DNA-RNA hybrid have in the cell? Perhaps helicase II helps to ensure the efficient release of RNA transcripts from the DNA template. In this regard it is known that the transcription termination factor Rho is an RNA-DNA helicase that may function to aid in the release of RNA transcripts (3). There are several notable differences between the hybrid unwinding reactions catalyzed by helicase II and Rho protein. (i) Helicase II unwinds long regions of DNA-RNA hybrid. Results presented here demonstrate unwinding of at least 250 bp of DNA-RNA hybrid; longer hybrid substrates have not been tested. The action of Rho protein is apparently confined to short duplex regions (3). (ii) Rho protein is thought to bind single-stranded RNA and move into the duplex region. The opposite appears to be true of helicase II, which binds DNA and moves into the duplex region. RNA fails to serve as the required nucleic acid cofactor in ATP hydrolysis reactions, suggesting that helicase II does not interact with single-stranded RNA (13, 14, 21). For this reason it seems unlikely that helicase II binds to the single-stranded RNA tail present on the hybrid substrate (see Fig. 1A). (iii) Rho protein requires a specific RNA sequence in order to initiate an unwinding reaction at a Rho-dependent terminator (3). Whether or not helicase II requires a specific sequence to initiate a DNA-RNA unwinding reaction is unknown. Other DNA-RNA hybrids composed of different sequences have not yet been tested. (iv) Rho protein unwinds RNA-DNA or RNA-RNA hybrids (3) whereas helicase II unwinds DNA-RNA and DNA-DNA hybrids.

Helicase II and Rho protein could act in concert (or separately) to aid in the displacement of RNA transcripts from the DNA template. The binding affinity of the two proteins for their respective nucleic acid and their opposite unwinding polarities (3, 22) would be consistent with the two enzymes working together. In this regard the phenotype of uvrD–rho double mutants might be instructive. Although rep–rho double mutants are apparently not viable (33), I am not aware of any data regarding the phenotype of uvrD–rho double mutants.

Alternatively, helicase II could be involved in unwinding the RNA component of Okazaki fragments on the lagging-strand side of the replication fork. The polarity of the helicase II unwinding reaction (22) is consistent with this idea. Such a role might be relevant to the observation that uvrD–polA double mutants are not viable (34–36). Presumably, DNA polymerase I removes the RNA primer on the Okazaki fragment by using its 5' → 3' exonuclease, although perhaps inefficiently when helicase II is present. In the absence of DNA polymerase I, helicase II could unwind the DNA-RNA duplex at the 5' end of the Okazaki fragment and allow DNA polymerase III to fill in the gap prior to ligation. In this case a nuclease activity must be available to excise the unwound RNA primer.

Yet another possibility is that helicase II acts to move transcribing RNA polymerase molecules from the path of the replication apparatus. Transcription occurs at a rate that is more than an order of magnitude slower than DNA replication (37). Bedinger et al. (37) showed that the phage T4 DNA replication complex slows to the rate of the transcribing RNA polymerase when "trapped" behind an RNA polymerase molecule. The phage T4 Dda protein, also a helicase, allowed the replication complex to move past the RNA polymerase and resume high rates of replication (37). Presumably, a DNA-RNA unwinding activity would facilitate such a process by ensuring unwinding of the nascent RNA strand. A similar role for helicase II in the cell has not been addressed experimentally and remains a possibility.

These suggestions for the role of helicase II in terms of its ability to unwind a DNA-RNA hybrid are speculative and must await further experimental testing. However, it seems likely that helicase II is involved in some aspect of nucleic acid metabolism, perhaps in a role not yet imagined, that requires the unwinding of DNA-RNA hybrids in the cell.

I thank J. W. George, K. A. Kaiser-Rogers, and Dr. B. K. Kay for critical reading of the manuscript and Susan Whitfield for preparation of the artwork. I am particularly grateful to Dr. B. K. Kay for suggesting this series of experiments. I gratefully acknowledge D. McCarn for preparation of helicase II, E. R. Wood for purification of helicase IV, E. E. Lahue for purification of helicase I, and J. E. Yancey for purification of Rep protein. This investigation was supported by National Institutes of Health Grant GM33476-04 and American Cancer Society Grant MV-332. S.W.M. is a recipient of an American Cancer Society Faculty Research Award.