Nucleic Acids Research, 1997, Vol. 25, No. 15 **2973–2978**

DNA helicase activity in Werner's syndrome gene product synthesized in a baculovirus system

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Received May 6, 1997 Revised and Accepted June 19, 1997

ABSTRACT

The gene responsible for Werner's syndrome (WRN) contains a region homologous to the *Escherichia coli* RecQ type DNA helicase and was thought to code for a DNA helicase belonging to this helicase family. However, no evidence has been shown before to substantiate this prediction. Here, we show data that the product of the WRN gene is indeed a DNA helicase. The gene product, a polypeptide with a relative molecular mass of 170 kDa, expressed in the insect *Spodoptera frugiperda* (Sf21) cell and purified by affinity column chromatography contained both the ATPase and DNA unwinding activities characteristic of DNA helicase. Expressions in Sf21, as well as in HeLa cells, showed that the WRN DNA helicase is exclusively transported to the nucleoplasm, which is consistent with its function in DNA metabolism. Our studies on strand displacement suggest that WRN helicase can unwind not only a duplex DNA, but also an RNA–DNA heteroduplex, while the latter reaction seems less efficient. Enzymological features learned from the purified WRN helicase are discussed with respect to the biological function, which remains to be clarified.

INTRODUCTION

Werner's syndrome (WRN) is a rare autosomal recessive genetic disorder causing premature aging accompanied by rare cancers (1–3). Patients with WRN have a short stature, juvenile cataracts, atrophy of the skin, greying and loss of hair, diabetes mellitus, arteriosclerosis, osteoporosis and neoplasia (4). Yu *et al*. (5) identified a gene responsible for WRN that encodes a protein with 1432 amino acids homologous to the *Escherichia coli* RecQ type DNA helicase (6) containing seven helicase motifs and a highly charged N-terminal domain. We and others have identified a total of 19 different mutations so far in Caucasian, Syrian and Japanese WRN patients (7–9). All these mutations were only in the coding region of almost the entire predicted helicase molecule. They apparently result in the premature termination of translation by generating nonsense codons.

The gene that causes Bloom's syndrome (BLM, characterized by genetic instability and a predisposition to cancer) was also found to belong to this DNA helicase gene family (10). Although its prediction was made by comparing the sequences and by searching for a homology in a database, no biochemical evidence has been shown before to substantiate that the products of these genes are indeed functional DNA helicases. In general, DNA helicases bind one of the strands of duplex DNA and unwind, migrating on a single-stranded DNA using energy from ATP hydrolysis. The DNA helicases have roles in various reactions involved in DNA metabolism, including replication, recombination, repair and transcription of DNA molecules (11). The two helicase-mediated genetic diseases, WRN and BLM, are apparently caused by chromosomal instability, a common characteristic symptom (12,13), resulting from malfunctioning of each respective helicase, although which biochemical reactions of DNA metabolism these helicases participate in are not understood.

Previously, Ma *et al.* (14) proved that the ERCC3 protein, which is a component of the transcription factor TFIIH complex and is defective in xeroderma pigmentosum group B cells, has DNA helicase activity by expressing the gene in a baculovirus system. To test if the WRN gene codes for a DNA helicase, we expressed the intact WRN cDNA in the insect *Spodoptera frugiperda* (Sf21) cells using a recombinant baculovirus system and purified the protein product. In this report, we show that the expressed WRN gene product is transported to the nucleoplasm of the insect Sf21. In addition, we show that the purified WRN gene product has an ATP hydrolysing activity and a DNA unwinding activity.

MATERIALS AND METHODS

Construction of the recombinant plasmid transfer vector and generation of the recombinant baculovirus containing WRN cDNAs

A full coding region of cDNA (4296 bp) was prepared by the polymerase chain reaction (PCR) using a mRNA of the fibroblast from a healthy individual who contained no WRN mutations. The transfer vector was constructed using the *Nco*I sites of expression vector pAcHLT-B (PharMingen). In brief, the coding region of WRN cDNA was inserted downstream of the polyhedrin promoter of the baculovirus gene and the N-terminal hexa-histidine sequence to facilitate the purification of the expressed protein (15). The N-terminal region of recombinant protein was designed to contain a kinase site of protein kinase A (RRASVA)

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and a cleavage site of thrombin (LVPRGS) for labeling and further purification, respectively. After co-transfecting to Sf21 cells using a mixture of the transfer vector pAcHLT-HisWRN and the linearized AcNPV BaculoGold™ DNA (PharMingen) DNAs, the recombinant viruses were detected after 4–5 days in monolayer culture by visual plaque screening. The recombinant viruses were isolated after a series of analyses that included genomic analysis of the virus by PCR and protein analysis by immunoblotting using an antibody to an array of N-terminal histidines (Clontech). The WRN gene was expressed by infecting Sf21 cells with this recombinant virus at the multiplicity of infection of five and the cells were harvested after 2–3 days of culture in monolayer flasks or spinner bottles.

Purification of recombinant WRN gene product

The Sf21 cells after 3 days post-infection (p.i.) (~3×10^7 cells) were washed once with cold phosphate-buffered-saline, were pelleted by centrifugation (1000 *g* for 15 min at 4°C) and were stored at –80°C until used. To purify the protein product, the frozen cells were thawed and suspended in a hypotonic lysis buffer (1×10^7 cells/ml) containing 10 mM HEPES–KOH (pH 7.9), 1% NP-40, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$. The buffer also contained 1 mM each of protease inhibitors, including benzamidine hydrochloride, phenanthroline, aprotinin, leupeptin, pepstatin A and phenylmethanesulfonyl fluoride. After keeping on ice for 10 min, the mixture was centrifuged at 1000 *g* for 10 min to collect the nuclei. The nuclei in the pellet were resuspended by adding a lysis buffer containing 0.4 M NaCl, 15 mM HEPES–KOH (pH 7.9) and 5 mM $MgCl_2$ and the mixture was centrifuged at 12 500 *g* for 30 min at 4°C. The recombinant WRN gene product in the supernatant was applied to the Ni-nitrilo-tri-acetic acid agarose (NTA, Qiagen) column equilibrated with the same lysis buffer. The column was washed first with 50 mM HEPES–KOH (pH 7.9) buffer containing 300 mM NaCl, 10% glycerol, 40 mM imidazole and a mixture of protease inhibitors. It was further washed by 50 mM HEPES–KOH buffer (pH 6.0) containing 300 mM NaCl, 10% glycerol and protease inhibitors. The proteins bound to the column were eluted step-wise using 50 mM HEPES–KOH buffer (pH 6.0) containing 100, 200, 300, 400 and 500 mM imidazole and were characterized by 7% polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (SDS–PAGE) and by staining with Coomassie brilliant blue R-250. A prestained SDS–PAGE standard protein marker (Bio-Rad) was used for calibration of the molecular mass: it contained myosin (213 kDa), β-galactosidase (119 kDa), bovine serum albumin (83 kDa) and ovalbumin (47 kDa).

Expression of WRN gene in HeLa cells

Full-size cDNA was inserted into an expression plasmid pcDNA3 (Invitrogen) downstream of the human cytomegalovirus (CMV) promotor and the sequence coding for an N-terminal FLAG peptide with an amino acid sequence DYKDDDDK. Plasmid DNA was introduced into HeLa cells by lipofection with LIPOFECTAMINE™ Reagent (GibcoBRL) according to the manufacturer's instructions and was expressed transiently in the HeLa cells for 48 h. The transfected HeLa cells were monitored for expressed WRN protein by immunofluorescent microscopy using an antibody specific to the FLAG peptide (Kodak).

Antibody specific to WRN gene product

Rabbit polyclonal antibodies were prepared by injecting a peptide TETSSAERKRRV corresponding to the amino acid residues 1396–1407 of the predicted coding region of the WRN gene product after coupling with keyhole limpet hemocyanin.

ATPase assay

The standard reaction mixture (50 µl), containing 50 mM Tris–HCl (pH 7.5), 20 mM 2-mercaptoethanol (2-ME), 2 mM ATP (containing 1 µCi [γ-^{32}P]ATP or [α-^{32}P]ATP), 5 mM $MgCl_2$, 100 µg/ml calf thymus DNA and 0.5 mg/ml of bovine serum albumin (BSA), was incubated with the purified WRN gene product (~0.1–0.8 µg) at 37°C for 60 min. The reaction products, ADP and the inorganic phosphate (Pi) released from ATP, were separated by polyethyleneimine (PEI) thin layer ascending chromatography with 0.7 M LiCl as a developing solvent. The amounts of products were determined by measuring the radioactivities of the products separated on the PEI thin layers (16).

DNA helicase assay

The helicase activity was measured by displacing a radiolabelled DNA fragment from a partially duplex substrate. The substrate–DNA complexes consisting of a M13mp18 single-stranded circular DNA and a 5′-^{32}P-labelled complementary oligonucleotide DNA (or an RNA) was prepared by annealing the two components by the procedure of Ma *et al.* (14). A total of four different DNA and RNA oligonucleotides (24–60mer) were used to construct various types of DNA substrates. The sequences of the oligonucleotides were 24mer DNA (5′-CGC CAG GGT TTT CCC AGT CAC GAC), 40mer DNA (5′-GTC GAC TCT AGA GGA TCC CCG GGT ACC GAG CTC GAA TTC G), 60mer DNA [5′-$(A)_{10}$-GTC GAC TCT AGA GGA TCC CCG GGT ACC GAG CTC GAA TTC G-$(A)_{10}$] containing two non-complementary stretches of $(A)_{10}$ at the 5′ and 3′ ends and 18mer RNA (5′-CAG GGU UUU CCC AGU CAC). These oligonucleotides (1 pmol each) were labelled at the 5′ termini by incubating with [γ-^{32}P]ATP and T4 polynucleotide kinase. The labelled oligonucleotides were annealed with 2.5 µg of single strand circular M13mp18 DNA (TaKaRa) in 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl and 1 mM DTT by heating at 95°C for 4 min, keeping the mixture at 67°C for 60 min and then cooling slowly to 37°C. Non-hybridized oligonucleotides were removed by spin-filtration through a Sephacryl S-400 HR column (Pharmacia).

The labelled DNA substrate (~2.5 pg) was incubated at 37°C for 60 min with the purified WRN gene product (0.1–0.8 µg) in the reaction mixture (20 µl) containing 50 mM Tris–HCl buffer (pH 7.5), 1 mM $MgCl_2$, 0.5 mg/ml BSA and 2 mM 2-ME. The reaction products were analyzed by agarose gel electrophoresis, which separated the oligonucleotide released from the duplex DNA (or RNA–DNA heteroduplex) complexes. The gel was dried in a vacuum and was exposed to Amersham Hyperfilm with an intensifying screen for autoradiography. The radioactivity of the released oligonucleotides was also quantified by excising the bands from the dried gel and by measuring the radioactivity using a Beckmann scintillation counter.

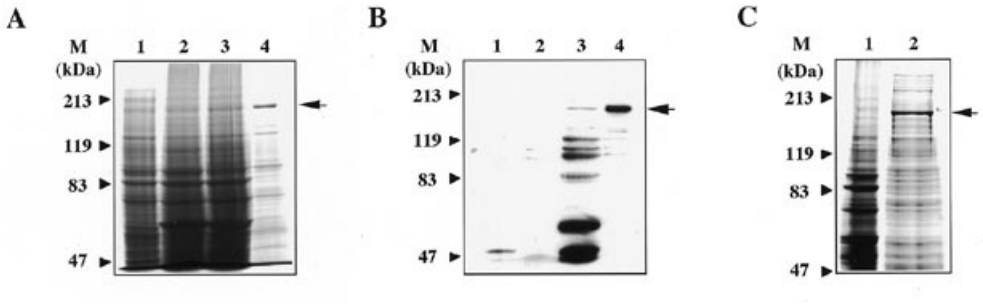

Figure 1. Expression of the WRN gene in insect Sf21 cells. (**A**) Analysis of whole cell lysates by SDS–PAGE. M-marker proteins: lane 1, uninfected Sf21 cell; lane 2, wild type AcNPV-infected cell; lane 3, WRN recombinant virus-infected cell; lane 4, partially purified cell lysate from WRN recombinant virus-infected cells (the cell lysate was mixed with NTA resin and the bound proteins were eluted by 500 mM imidazole after washing the resin with 50 mM HEPES–KOH buffer containing 40 mM imidazole and protease inhibitors). (**B**) Immunoblot analysis of whole cell lysates. Cell lysates resolved by SDS–PAGE in (A) were analyzed by immunoblotting using the WRN specific antibody. Arrangements of the lanes are the same as (A). (**C**) Subcellular distribution of the WRN gene product in Sf21 cells. The infected cells (2 days p.i.) were lysed as described in Materials and Methods and were fractionated into cytoplasmic and nuclear fractions. Protein in these fractions was analyzed by SDS–PAGE. M-marker proteins: lane 1, cytoplasmic fraction; lane 2, nuclear fraction. An arrow indicates the 170 kDa WRN gene product.

RESULTS

Expression of the WRN gene in insect Sf21 and HeLa cells

The WRN gene was expressed in Sf21 cells by infecting the cells with a recombinant baculovirus containing the full-size WRN cDNA. The lysates of the infected cells and a fraction of the lysate that bound to the Ni-NTA resin were analyzed by SDS–PAGE and by staining with Coomassie brilliant blue (Fig. 1A, lanes 3 and 4). A set of molecular mass markers were included in the electrophoresis as standards for calibration. The lysates of uninfected cells (lane 1) and the cells infected with wild baculovirus (lane 2) were similarly analyzed as controls. Simultaneously, the same samples were analyzed by immunoblotting (Fig. 1B) using antibodies prepared from the C-terminal peptide of the predicted coding region of WRN gene. In the immunoblotting, the lysates from the recombinant virus-infected cells (Fig. 1B, lane 3) showed a band with an approximate relative mass of 170 kDa (marked by an arrow head) that is close to the molecular weight of 168 000 Da deduced from the open reading frame of the WRN gene sequence and an extra N-terminal sequence (52 amino acid residues), including hexahistidine, protein kinase A and thrombin cleavage sites. The smaller polypeptides that reacted with the antibodies may be the proteolytic degradation products of the 170 kDa protein. The 170 kDa band was not easily visible in the stained gel, but when the whole lysate of the recombinant virus-infected cells was purified by affinity chromatography, a clear band was visible by SDS–PAGE (Fig. 1A, lane 4), which reacted with WRN specific antibodies during the immunoblotting (Fig. 1B, lane 4). This 170 kDa protein was found mostly in the nuclei of Sf21 cells when their subcellular distribution was examined (Fig. 1C). These results indicate that the WRN gene product is synthesized to its full size and is transported into the nucleoplasm.

To determine if the WRN gene product is also transported to nuclei in the mammalian system, the same cDNA was expressed in HeLa cells by an expression vector pcDNA3 designed to yield a putative WRN helicase with an N-terminal FLAG peptide. With this FLAG peptide, the location of expressed protein can be detected by immunofluorescent microscopy with the monoclonal antibody specific for the FLAG peptide. The result clearly shows that the FLAG-tagged WRN gene product is exclusively transported to the nucleoplasm of HeLa cells (Fig. 2).

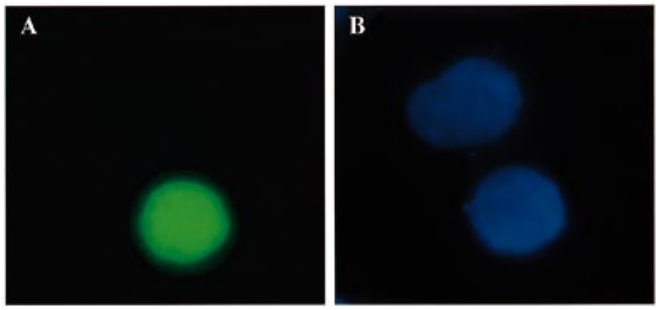

Figure 2. Nuclear localization of WRN gene product in HeLa cell. Full-size cDNA of the WRN gene was inserted into the *Kpn*I and *Xho*I sites of the expression vector pcDNA3 downstream of the CMV promotor and the FLAG sequence. The plasmid DNA was transfected transiently by lipofection with Lipofectamine™ Reagent (GibcoBRL) following the instructions of the manufacturer. (**A**) The transfected cells were cultured for 48 h at 37°C, fixed on a glass plate and were reacted with anti-FLAG monoclonal antibodies. The expressed protein was visualized by staining with anti-mouse antibodies labelled with FITC and was monitored by fluorescence microscopy. (**B**) The nuclei of the same cells in (A) were stained by a fluorescent dye DAPI, 5-(*N*-2,3-dihydroxypropylactamido)-2, 4, 6-triiodo-*N*,*N*′-(bis2, 3-dihydroxypropyl)isophthalamide.

Purification of the WRN gene product from the nuclei of Sf21 cells

The WRN gene product expressed by the recombinant baculovirus was extracted from the nuclei of Sf21 cells 2–3 days p.i. and was purified by Ni-NTA affinity column chromatography. Due to the N-terminal hexahistidine tag, the WRN gene product bound to the Ni-NTA resins was recovered by stepwise elution with increasing concentrations of imidazole. Analysis of the proteins by SDS–PAGE showed that a protein with a molecular mass of 170 kDa was eluted mainly by high concentrations (300–500 mM) of imidazole (Fig. 3A, lanes 5–7), while this protein was not visible in the 0 mM (lane 1), the 40 mM (lane 2), the 100 mM (lane 3) and the 200 mM imidazole fractions (lane 4). To distinguish the expressed WRN gene product from the insect cell proteins, a cell lysate prepared similarly from the cells

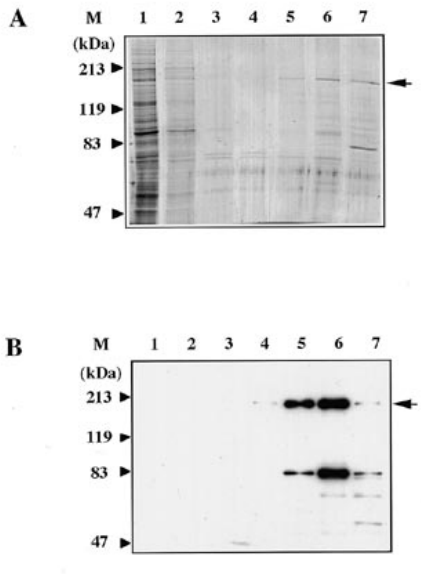

Figure 3. Purification of the WRN gene product by Ni-NTA column chromatography. The nuclear lysate was prepared from WRN recombinant virus-infected cells and the WRN gene product was purified by Ni-NTA column chromatography as described in the Materials and Methods. Proteins eluted by the pH 6.0 buffer containing 0–500 mM imidazole were analyzed by (**A**) SDS–PAGE and (**B**) immunoblotting. M-marker proteins: lane 1, buffer alone; lane 2, 40 mM imidazole; lane 3, 100 mM imidazole; lane 4, 200 mM imidazole; lane 5, 300 mM imidazole; lane 6, 400 mM imidazole; and lane 7, 500 mM imidazole.

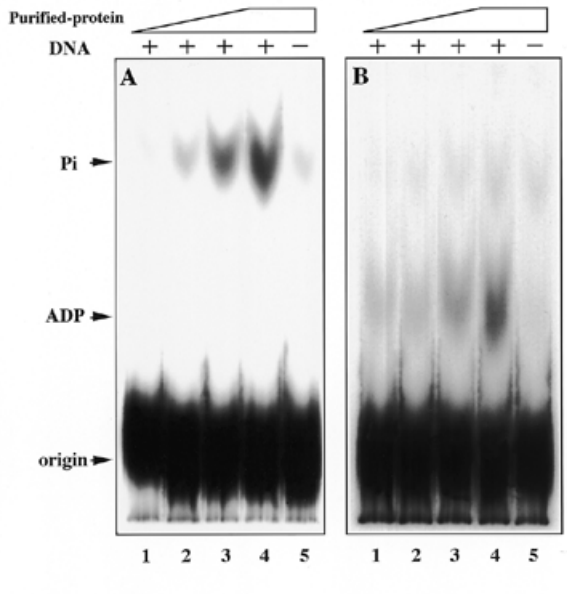

Figure 4. Determination of ATPase activity associated with the WRN gene product. Pooled 400 and 500 mM fractions shown in Figure 3 were examined for ATPase activity by PEI thin layer chromatography. (**A**) The increasing amounts of WRN gene product purified by Ni-NTA resin were incubated with [γ-^{32}P]ATP under the conditions described in Materials and Methods. (**B**) Same as (A), except [α-^{32}P]ATP was used as the substrate.

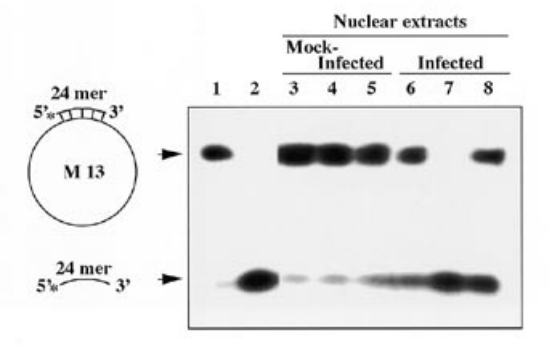

Figure 5. Characterization of DNA helicase activity associated with the purified WRN gene product. DNA helicase activity was analyzed for the nuclear extracts of infected Sf21 cells fractionated by Ni-NTA column chromatography. The eluate fractions with 300, 400 and 500 mM imidazole were used in this assay. As controls, the same fractions obtained from mock-infected Sf21 cells were similarly tested, using the ^{32}P-labelled 24mer DNA oligomer–M13 DNA complex as the substrate. Lanes 1 and 2 represent the substrate DNA and the heat-denatured substrate DNA, respectively. Lanes 3, 4 and 5 represent the reaction products obtained with 300, 400 and 500 mM imidazole fractions, respectively. Lanes 6, 7 and 8 show the results with 300, 400 and 500 mM imidazole fractions from infected Sf21 cells, respectively. The products were separated by agarose gel electrophoresis. A schematic diagram of the substrate structure is shown.

infected with the wild type virus was subjected to the same affinity column chromatography as the control. However, no protein band migrating with the same molecular mass of 170 kDa was in the 400 and 500 mM imidazole fractions (data not shown). The immunoblot analysis with an antibody specific to hexahistidine showed that the proteins reactive to the antibody were from the 300–500 mM imidazole fractions and are split into two fractions, 170 and 80 kDa polypeptides. The latter polypeptide is perhaps the C-terminal truncated product generated by proteolytic degradation of the 170 kDa protein during the purification steps. These results suggest that the 170 kDa protein eluted by high concentrations of imidazole was the WRN gene product.

Characterization of enzymatic activity of the purified WRN gene product

ATPase activity. The purified WRN gene product was examined for its potential helicase activity, particularly the ATPase and the DNA unwinding activities. First, the ATPase activity was measured with increasing amounts of pooled 400 and 500 mM imidazole fractions. After incubating with [γ-^{32}P]ATP, the reaction products were analyzed by PEI-thin layer chromatography (Fig. 4A, lane 1). The radiolabelled phosphate in the ATP was released as Pi. Similar experiments with [α-^{32}P]ATP yielded [^{32}P]ADP, but no [^{32}P]Pi (lane 2), indicating that the activity contained in the purified WRN product is a γ-ATPase that removes only the γ-phospate of ATP. This ATPase activity was dependent on the presence of DNA and no activity occurred without adding DNA (lanes 5 of Fig. 4A and B). When the same eluate fraction obtained from the cells infected with wild type baculovirus was tested, no ATPase activity was detected (data not shown). These results indicate that the ATPase activity is associated with the expressed WRN gene product. A preliminary

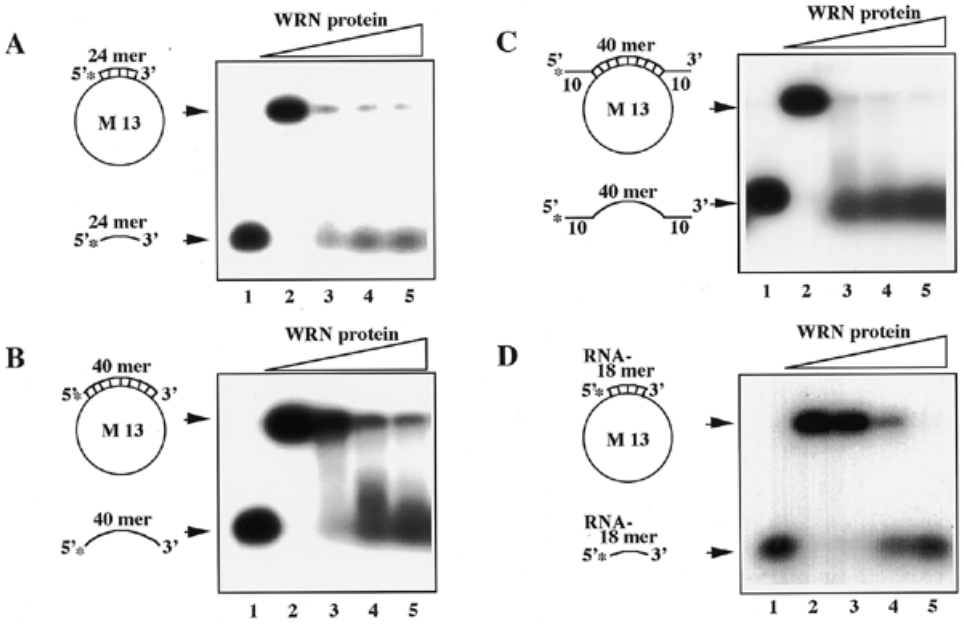

Figure 6. Unwinding reactions catalyzed by the purified WRN helicase. The unwinding activity of WRN DNA helicase was evaluated with four different types of substrates as described in Materials and Methods. The reaction products were separated by agarose gel electrophoresis and were analyzed by autoradiography. A schematic diagram of the structure of each substrate is shown.

kinetic study showed that the ATPase associated with the WRN gene product can hydrolyze ATP at a rate of 6.7 μmol/μg protein/min under the standard conditions.

DNA helicase activity. Helicase activity was tested with the WRN gene product using various types of ^{32}P-labelled substrates. First, the substrate DNA consisting of 5′-^{32}P-labelled oligodeoxynucleotide (24mer) and the single-stranded M13 DNA was incubated with proteins eluted from the Ni-NTA column. The 5′-labelled short DNA released from the duplex DNA was monitored by agarose gel electrophoresis (Fig. 5). The released short single-stranded DNA migrated fast in the gel, while the original duplex DNAs with a high molecular weight migrated slowly near the origins of the gel. The proteins from infected Sf21 cells that were eluted with high imidazole concentrations (300, 400 and 500 mM) showed helicase activity, with the highest activity in the 400 mM fraction (Fig. 5, lane 7). Relatively low activities in the 300 and 500 mM fractions (Fig. 5, lanes 6 and 8) were proportional to the amounts of 170 kDa WRN gene product estimated from the results with immunoblotting (Fig. 3B, lanes 5 and 7). By contrast, no DNA helicase activity was detected in the same fractions prepared from the mock-infected cells (Fig. 5, lanes 3–5). These results clearly demonstrated that DNA helicase activity occurs within the WRN gene product expressed in the insect cells.

Characterization of the DNA unwinding reaction by WRN DNA helicase. To further characterize the helicase activity, a series of experiments were carried out using the purified WRN DNA helicase (a pooled fraction of imidazole 400 and 500 mM). Figure 6 shows schematically the substrate DNAs used in these experiments. Figure 6A shows that the DNA unwinding reaction is dependent on the amount of WRN DNA helicase, with a complex consisting of 5′-^{32}P-labelled oligodeoxynucleotide (24mer) and M13 DNA as the substrate. Curiously, we noted that the intensity of the oligonucleotide band decreased compared to the starting material not exposed to the WRN helicase preparation, while the reason behind this observation is unclear as we discuss later. When the duplex region increased using a longer ^{32}P-labelled oligodeoxynucleotide (40mer), the WRN DNA helicase was still able to remove this oligonucleotide, although the rate of reaction apparently decreased and the released oligonucleotides retarded the migration at gel electrophoresis for unknown reason (Fig. 6B). However, when two unpaired 10mers were added to this oligodeoxynucleotide at its 3′ and 5′ ends, the unwinding reaction by WRN DNA helicase increased to the equivalent level obtained with a short 24mer or to a higher level (Fig. 6C). These results suggest that the WRN DNA helicase perhaps has a greater affinity for the single-stranded region and starts the unwinding of the duplex DNA structure from single-stranded regions. The ribonucleotide (18mer) in the RNA–DNA heteroduplex molecule with M13 DNA could also be released by WRN DNA helicase (Fig. 6D), while a greater amount of enzyme was needed compared to the reaction with the oligodeoxynucleotide (Fig. 6A). The result suggests that the WRN DNA helicase can unwind not only the DNA duplex, but also the RNA–DNA heteroduplex.

DISCUSSION

In this study, we proved that the WRN gene, which had been thought to code for DNA helicase from a nucleotide homology search, indeed encodes a DNA helicase and by expressing the gene in an insect cell, we purified the protein from cell lysates and

characterized the enzymatic activities associated with the protein. The product of WRN DNA helicase has an apparent molecular mass of 170 kDa and has an ability to hydrolyse ATP and to unwind duplex DNA. In addition, we found that the expressed WRN helicase was transported to the nucleoplasm of insect cells. A simultaneous expression study with HeLa cells showed that the WRN DNA helicase is also transported to the nucleoplasm, consistent with its biological function in the DNA metabolism in cell nuclei. In our study, the ATPase activity associated with WRN helicase was dependent on the presence of DNA, similar to a previous finding with RecQ1 DNA helicase (17). The helicase activity seems to be influenced by the structure of substrate DNA (Fig. 6). For instance, the short DNA fragment appears to be more easily unwound and displaced than the longer fragments. Also, the DNA fragment with single-stranded ends is presumably a better substrate for the unwinding reaction (Fig. 6B and C). The WRN helicase having an ability to unwind RNA molecules hybridized to DNA is noteworthy, while the efficiency in this activity is low compared to that with a homoduplex DNA molecule. In Figure 6A, a significant decrease in the intensity of the released oligonucleotide band was observed compared to the starting material, suggesting that a part of the oligonucleotide was degraded by the exposure to the WRN helicase preparation. A similar reduction was also found for the released RNA oligonucleotide in Figure 6D (lane 5). A more quantitative study is needed to clarify if the released oligonucleotides are digested by the intrinsic nuclease activity of the WRN helicase or by the insect cell nuclease(s) that might contaminate the WRN helicase preparation. In this context, Lombard and Gurante (18) previously predicted that the WRN helicase may contain an N-terminal nuclease domain homologous to bacterial RNase D and a 3′–5′ proofreading exonuclease domain of bacterial DNA polymerase I (PolA). Also, Mushegian *et al*. (19) reiterated recently this prediction for the N-terminal globular domain of WRN helicase after making computational homology searches; they suggested that the combined domains of predicted nuclease and helicase may be involved in DNA repair or RNA processing. The availability of enzymatically active WRN helicase will now permit the further characterization of its biochemical properties and functions.

Consistent with the high homology between human RecQ1 and WRN DNA helicase genes, the enzymatic features of WRN DNA helicase are virtually similar to RecQ1 helicase (16,17), while the molecular mass of the latter is small (73 kDa). Although both helicases share similarities in their *in vitro* biochemical activities, their biological roles *in vivo* seem to be dissimilar judging from the expression profiles of the genes. The RecQ1 helicase gene is expressed ubiquitously in all the organs and tissues thus far tested, while the expression of WRN helicase gene is rather organ-specific: high in testis, ovary and pancreas and low in lung, brain, kidney and leukocytes (5; Shimamoto *et al*. unpublished results). The defective WRN gene causes premature aging and abnormal cancers (3), whereas no disease has been reported in relation to the human RecQ1. Mutation(s) in the RecQ1 gene may possibly have a lethal effect on embryos during the early stage of development, because of the gene expression in many organs.

The existence of multiple species of RecQ type DNA helicase in human cells suggests that the biological role of the helicases in this particular RecQ family are divergent in human cells, although *E.coli* and yeast cells contain only one RecQ type DNA helicase, RecQ and sgs1, respectively. Perhaps, each of human RecQ type helicases may be involved in a specific task in DNA metabolism and the function in specific organs or cells, resulting in the differential clinical phenotypes when defective. In this context, further studies are needed to clarify in what biological reactions or in what kind of DNA structure these DNA helicases participate or unwind.

ACKNOWLEDGEMENTS

We thank Drs Minoru Sugawara and Masanobu Sugimoto of AGENE Research Institute for valuable discussions and encouragements. We also gratefully acknowledge Dr Mitsuo Oshimura of the Tottori University, Faculty of Medicine, for critical comments prior to publication and helpful discussions. This work was supported by the Drug Organization (The Organization for Drug ADR Relief, R & D Promotion and Product Review, supervised by the Ministry of Health and Welfare of Japanese Government).

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