[5] Analysis of the DNA Unwinding Activity of RecQ Family Helicases

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Abstract

The RecQ family of DNA helicases is highly conserved in evolution from bacteria to mammals. There are five human RecQ family members (RECQ1, BLM, WRN, RECQ4 and RECQ5), defects, three of which give rise to inherited human disorders. Mutations of *BLM* have been identified in patients with Bloom's syndrome, *WRN* has been shown to be mutated in Werner's syndrome, while mutations of *RECQ4* have been associated with at least a subset of cases of both Rothmund-Thomson syndrome and RAPADILINO. The most characteristic features of these diseases are a predisposition to the development of malignancies of different types (particularly in Bloom's syndrome), some aspects of premature aging (particularly in Werner's syndrome), and on the cellular level, genome instability. In order to gain understanding of the molecular defects underlying these diseases, many laboratories have focused their research on a study of the biochemical properties of human RecQ helicases, particularly those associated with disease, and of RecQ proteins from other organisms (e.g., Sgs1p of budding yeast, Rqh1p of fission yeast, and RecQ of *E.coli*). In this chapter, we summarize the assay systems that we employ to analyze the catalytic properties of the BLM helicase. We have successfully used these methods for the study of other RecQ and non-RecQ helicases, indicating that they are likely to be applicable to all helicases.

Introduction

Helicases are motor proteins that couple the hydrolysis of nucleoside triphosphate (usually ATP) to the breakage of the hydrogen bonds between the complementary bases of duplex DNA. This process is often referred to as DNA unwinding or DNA strand separation. DNA helicases function in all aspects of DNA metabolism where they are required to permanently or transiently unwind regions of the genome to permit, for example, initiation of DNA replication or transcription, or the execution of DNA repair. Because of their roles in a diverse range of processes, many

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helicases have become highly specialized to a particular step in a single key process (such as the *E. coli* DnaB helicase that unwinds the DNA ahead of the translocating replication fork), while others play roles in more than one cellular process (such as the XPB and XPD helicases that function in nucleotide excision repair and in transcription as components of TFIIH). Based on comparisons of primary sequence, DNA helicases have been classified into distinct superfamilies (Singleton and Wigley, 2002). Superfamily I and II represent the bulk of the known helicases. Representatives of these superfamilies contain a conserved helicase domain that comprises seven characteristic sequence motifs. In contrast, the superfamily III and IV helicases contain three and five of these signature motifs, respectively. In all cases, two of these motifs represent the so-called Walker A and B boxes that are essential for NTP binding and hydrolysis. Members of the RecQ family of helicases (Bachrati and Hickson, 2003) contain all seven of the signature motifs, as well as a number of other conserved stretches of sequence that distinguish them from other superfamily I and II members.

Preparation of Substrates

RecQ helicases have been shown to be able to be capable of unwinding a wide array of DNA duplex and partial duplex substrates (Bachrati and Hickson, 2003). Although these structures are designed to model many different putative *in vivo* DNA substrates for these helicases and may appear superficially to be very different, in fact they can be created using just a few standard procedures. Following initial preparation of these substrates, it is essential that they be purified to remove nonincorporated precursor molecules or the by-products of synthesis, which might serve as binding competitors.

Substrates Prepared from Annealed Oligonucleotides

The main advantage of oligonucleotide-based substrates is the ease with which they can be generated. Some of these substrates are simple DNA structures; however, many of them have been designed to model a particular *in vivo* DNA structure such as a replication fork or a Holliday junction recombination intermediate (Fig. 1). Nevertheless, one must be aware of the limitations of these model structures, as they may either have features not existing *in vivo* or lack features of the genuine *in vivo* substrate.

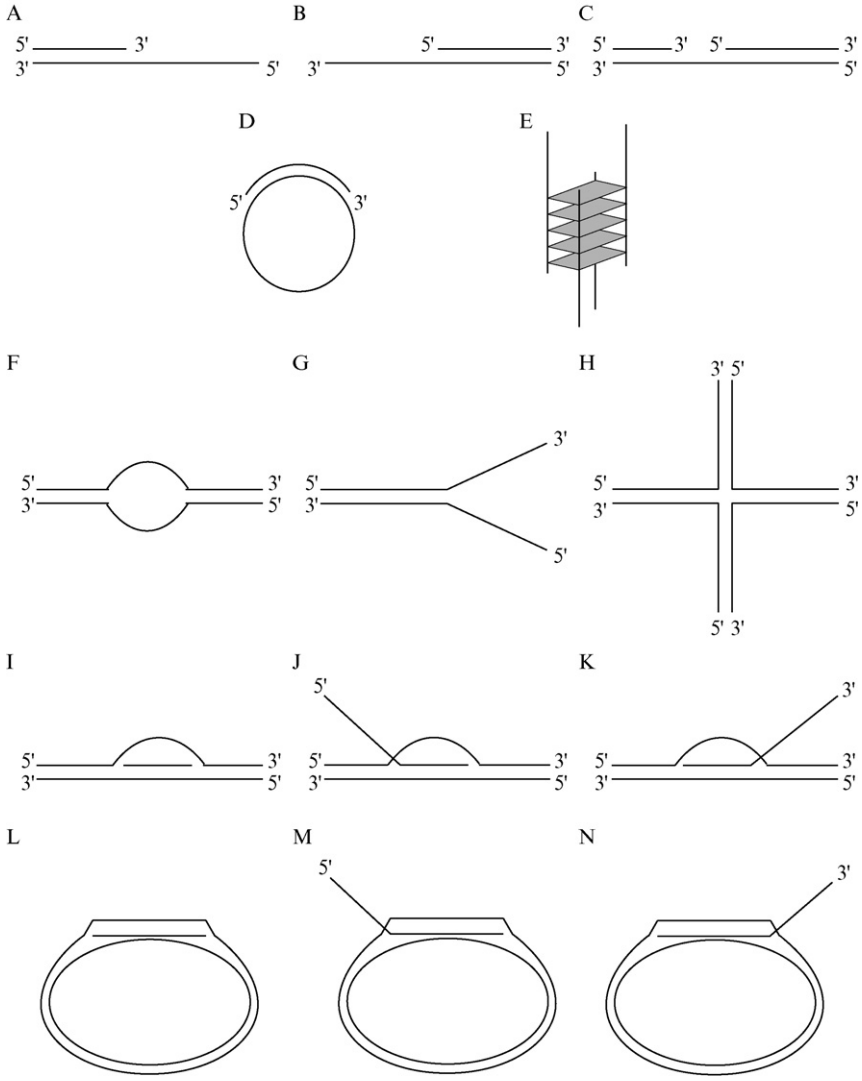

FIG. 1. Schematic representation of the most commonly used helicase substrates. (A) and (B) Partial duplexes with 5′ and 3′ protruding ends, respectively. (C) Partial duplex with a gap in the middle; commonly used to determine the orientation of helicase unwinding. (D) Partial duplex made by annealing a synthetic oligonucleotide to the complementary sequences of a single-stranded circular dna, such as X174 or m13 bacteriophage. (E) G4 quadruplex DNA. (F) ''Bubble.'' (G) Replication fork. (H) Synthetic Holliday junction. (I–K) Oligonucleotide-based, static D-loops. (L–N) Plasmid-based, mobile D-loops.

Detection of reaction products in helicase assays is generally based on the incorporation of radiolabel into one or more of the oligonucleotides that comprise the substrate. Generally speaking, only one of the oligonucleotides is labeled and its release from the intact substrate is monitored. The oligonucleotides must be of the highest quality and homogeneous in terms of length in order to obtain interpretable data. This is best achieved by PAGE purification, a detailed method which can be found in several standard laboratory manuals. Current Protocols in Molecular Biology (2005) is our preferred text.

Procedure

1. To generate 5′ ^{32}P-labeled oligonucleotides, incubate 10 pmol of oligonucleotide with T4 polynucleotide kinase and [-^{32}P]ATP using the forward reaction protocol. If it is necessary to label the 3′ end of the oligonucleotide, use terminal deoxynucleotidyl transferase and [-^{32}P]-ddATP (N.B. an extra nucleotide will be added). Separate the unincorporated nucleotides from the oligonucleotide on a Sephadex G50-based microcentrifuge spin column (such as the mini Quick Spin Columns of Roche Diagnostics, Indianapolis, IN), keeping the elution volume low (<30 l). Measure the radioactivity of the labeled oligonucleotide and determine its specific activity assuming an 95% DNA recovery from the column.

2. In a screw-capped microcentrifuge tube on ice containing the labeled, purified oligonucleotide solution, add the remaining reaction components in the following order: 5 l 100 m*M* Tris-HCl, pH 7.5; 5 l 100 m*M* $MgCl_2$ and 30 pmoles each of the other oligonucleotides. Bring the volume up to 50 l with ddH_2O.

3. It is not necessary to have any specialist equipment to set up the annealing of oligonucleotides; this can be done with ''homemade'' equipment. Heat up an almost full 2-liter glass beaker of water to 95 , and carefully place the beaker into a polystyrene box that can easily accommodate it. Place the tube with the annealing mixture into a polystyrene tube rack, and float this on the surface of the hot water. Close the polystyrene box with its lid; it will provide adequate insulation to allow the water to cool down slowly over several hours.

Sometimes it is necessary to anneal more than two oligonucleotides in a particular order. In this case, the incubation and the controlled cooling of the annealing mixture are most conveniently carried out in an ordinary PCR machine (thermal cycler) (Opresko *et al.*, 2004).

1. Program your PCR machine as follows: hold at 95 for 5 min; cool down to 60 at 1.2 /min; hold at 60 for 60 min; and cool down to 20 again at 1.2 /min. Some PCR machines cannot be programmed to such slow automatic cooling rate; stepwise cooling can work equally well.

2. In a 200- l thin-walled PCR tube set up the mixture as stated previously, but containing only the first two of the multiple oligonucleotides to be annealed. Put into the PCR machine and start the program described in step 1. above. When the temperature reaches 60 , add the third oligonucleotide and continue the program.

At the completion of the annealing process continue to substrate purification.

Generation of Displacement Loop (D-loop) Substrates with RecA-Mediated Strand Invasion

In order to gain a more accurate picture of the function of helicases, one should consider using substrates *in vitro* that model the *in vivo* DNA structures as closely as possible. One such example is the use of plasmid-based displacement loop (D-loop) structures generated by RecA-mediated strand transfer, instead of oligonucleotide-based D-loops that have the same sequence. These D-loop substrates represent the initial stage of homologous recombination reactions. The plasmid-based D-loop substrates can be built according to the method of McIlwraith *et al.* (McIlwraith *et al.*, 2001). The invading radiolabeled oligonucleotide is coated with RecA molecules in the first step and then a supercoiled plasmid is added. RecA facilitates a homology search between the plasmid and the oligonucleotide and catalyzes the invasion of the homologous single-stranded sequences into the plasmid while displacing the complementary strand of the plasmid. The invasion step is reversible and if the reaction is allowed to proceed too long, RecA catalyzes the removal of the invading oligonucleotide from the plasmid. The reaction must, therefore, be stopped at the point where the maximal level of D-loop has been generated. This point is best determined experimentally.

Materials

10 RecA buffer
500 m*M* triethanolamine-HCl, pH 7.5
150 m*M* $MgCl_2$
10 m*M* DTT
1 mg/ml BSA.

Procedure

1. In a screw-capped microcentrifuge tube, mix the following:

   10.68 l 10 RecA buffer
   12.0 l 200 m*M* phosphocreatine (in 1 RecA buffer)
   1.2 l 200 U/ml creatine phosphokinase (in 1 RecA buffer)
   2.4 l 100 m*M* ATP
   360.0 pmol (nucleotides) radiolabeled oligonucleotide.

2. Bring up the volume with ddH_2O such that, when the further components of the reaction mix are added (see later), the final volume will be 120 l.

3. Add 480 pmol RecA (using the 2 mg/ml RecA product supplied by New England BioLabs, Beverly, MA; this will be 9.08 l), mix well and incubate at 37 for 5 min.

4. Add supercoiled plasmid DNA (ideally purified with two consecutive runs of ethidium-bromide saturated CsCl equilibrium gradient ultracentrifugation) to the final concentration of 300 *M* (nucleotides), mix well and incubate at 37 for the optimal length of time determined previously.

5. Stop the reaction by the addition of 24 l of 100 m*M* Tris-HCl, pH 7.5, 100 m*M* $MgCl_2$, 3% SDS, and 10 mg/ml proteinase K. Mix well and incubate at 37 for a further 30 min to allow deproteinization.

Proceed to substrate purification.

Generation of G4 Quadruplex Substrates

G-DNA structures have been shown to form readily *in vitro* in certain oligonucleotides that contain runs of guanines, and are stabilized by Hoogsteen hydrogen bonding (Sen and Gilbert, 1988). Depending on strand stoichiometry, strand orientation, and the presence of monovalent cations, G-DNAs can exist in different forms. In the presence of Na^+ ions the preferred configuration is G4 DNA, formed from four parallel strands, while in the presence of K^+ ions the planar structure is formed between two fold-back strands (G2′DNA) (Sen and Gilbert, 1990). The *in vivo* presence of G-DNAs has not yet been proven conclusively, although transient formation of G-DNAs has long been suggested at the rDNA locus, telomeres, in the immunoglobulin heavy chain switch regions, and at the c-Myc promoter (Arthanari and Bolton, 2001). Members of the RecQ family of helicases have been shown to be capable of unwinding not only B-form DNA, but also G4 quadruplex DNA (Huber *et al.*, 2002; Li *et al.*, 2001; Sun *et al.*, 1998).

G4 quadruplex DNA substrates are formed from unlabeled oligonucleotides and purified in that form. The purified substrate is stable in storage buffer for several months and aliquots of it can be labeled as required.

Procedure

1. Set up 100 l of G4 oligonucleotide solution with oligonucleotide at 2 g/ l in 1 *M* NaCl.
2. Heat denature at 100 for 5 min and then incubate at 60 for 48 h.
3. Gel purify using the procedure for nonradioactive substrates (see later).
4. Precipitate the substrate by the addition of 1/10 volume of 3 *M* Na-acetate, pH 5.2 and 1 volume of isopropanol at 20 . Collect the precipitate by centrifugation in an Eppendorf microcentrifuge (or equivalent) for 5 min. Discard the supernatant.
5. Dissolve the pellet in 100 l of 100 m*M* NaCl, 10 m*M* Tris-HCl, pH 8, 1 m*M* EDTA.
6. Store at 20 .

Notes: Formation of G4 DNA is facilitated by high oligonucleotide and Na^+ concentrations. Even under these circumstances, there is an equilibrium between the G4 quadruplex form and the single-stranded oligonucleotide form of the substrate DNA. Though the G4 quadruplex is relatively stable, it is vital to carry out the electroelution step at 4 in order to keep the ratio between the G4 and single-stranded forms as high as possible.

Purification of Radioactive Substrates

In order to remove impurities that might adversely affect the helicase reaction, the substrate DNA must be gel purified. We have found that a good separation of full-length substrate and impurities can be achieved on 5% polyacrylamide gels. Moreover, because of the lower percentage of the gel, recovery of the DNA is more efficient.

Procedure

1. Add DNA gel loading dye to the product of the substrate generation reaction above and load into the wells of a 5% polyacrylamide TBE (Tris-Borate-EDTA) gel.

2. Run in 1 TBE buffer (90 m*M* Tris base, 90 m*M* boric acid, 2 m*M* EDTA, pH 8.0) at 4 . The running parameters depend on the size of gel used and the size of molecules to be separated; we usually run 12-cm long gels at 25 mA constant current for 60 min.

3. Remove one of the support plates and cover the gel in Saran Wrap.

4. Expose the gel to X-ray film for around 5 min in a dark room. Put markers on the X-ray film to allow alignment of the developed film and the gel. Develop the film.

5. Cut out the area of the film that corresponds to the radioactive substrate (there may be multiple bands present, the structurally intact substrate is usually the one with the slowest mobility), align the film with the gel, and use the film as a template to locate the area of the gel that contains the substrate. Cut the gel along the template ''window'' with a scalpel.

6. Lift the cut gel slice out of the gel and transfer it into a dialysis tube with 1 TBE for electroelution. We find that the electroelution cassettes of the Qbiogene (Irvine, CA) ProteoPlus kit are very handy for this purpose; the dialysis step can then be performed in the same chamber without the need to transfer the radioactive liquid into another dialysis bag.

7. Carry out the electroelution by running at constant voltage (usually 120 V for 2 h) in 1 TBE at 4 . At the end of electroelution, reverse the polarity of the current for 30 s. This step dissociates the DNA molecules from the inner wall of the dialysis tubing.

8. Rinse the inner wall of the dialysis tube several times with the liquid inside the chamber and transfer the liquid into a Slide-A-Lyzer dialysis cassette (Pierce Biotechnology, Rockford, IL) for dialysis.

9. Dialyze against 1000 ml of 10 m*M* $MgCl_2$ and 10 m*M* Tris-HCl, pH 7.5, for at least 2 h at 4 , changing the dialysis buffer every 20 min.

10. Transfer the dialyzed substrate into screw-cap microcentrifuge tubes and freeze in 100- l aliquots.

11. Measure the radioactivity of the purified substrate and, using the specific activity of the labeled oligonucleotide, calculate the chemical concentration of the substrate.

Notes: Although plasmid-based D-loop molecules are large and best purified from agarose gels, we usually carry out the purification on 5% polyacrylamide gels, as described previously, since detection of the radioactive DNA molecules and handling of the thinner acrylamide gel is more straightforward. Also, we found that DNA recovery methods from agarose gels often give rise to impurities that inhibit the helicase reaction, or cause the D-loop molecules to dissociate during the purification process. It should also be noted that the supercoiled plasmid, which is added at a large molar excess to the reaction, copurifies with the radioactive D-loop. As RecQ helicases do not unwind supercoiled double-stranded plasmid DNA molecules, in most cases this is not a problem.

Purification of Nonradioactive Substrates

As the G4 quadruplex substrate is generated from nonlabeled oligonucleotides, detection of the DNA for purification purposes is achieved through UV shadowing.

Procedure

1. Run the prepared substrate on a 5% polyacrylamide gel, as described previously.
2. Cover a thin layer chromatography (TLC) plate, which contains a UV fluorescent dye for detection (such as the POLYGRAM CEL 300 PEI/UV_{254} of Macherey-Nagel, Germany) in Saran Wrap.
3. Remove one of the supporting plates from the gel and lay the gel onto the TLC plate. Remove the top supporting plate and cover the whole assembly in Saran Wrap. (N.B. the gel might have flipped during this, reversing the loading order.)
4. In the dark room, use a handheld UV lamp to expose the gel/TLC plate assembly briefly to short wavelength UV light (254 nm). As DNA molecules absorb UV light, the band in the gel where the DNA is present should make a shadow on the fluorescing TLC plate. Mark the position of the band with a marker pen. UV exposure must be for as brief a period as possible, in order to avoid damaging the DNA. If there are multiple bands on the same gel to be isolated, it is a good practice to cover the lanes that are not being processed immediately with a glass plate for protection.
5. Cut out the marked gel slice with a scalpel, and continue with electroelution and dialysis as described previously.

Analysis of Helicase Activity

The Assay System

Buffer Composition: RecQ helicases display two enzymatic activities: Mg^{2+} and DNA-dependent ATPase activity, and ATP-dependent helicase activity. Although the ATPase activity requires DNA as a cofactor, unwinding of the DNA is not a prerequisite for ATP hydrolysis. Hence, ATP hydrolysis can be uncoupled from DNA unwinding. The reaction buffer must, therefore, contain ATP and Mg^{2+}, as well as the DNA substrate. The optimal buffer composition must always be determined experimentally; the following guidelines might serve as a starting point:

1. The optimal pH for the reaction is generally around 7.5 to 8.0, which can be accomplished by the use of Tris-based buffers. Helicases that prefer neutral conditions are best analyzed in HEPES-based buffers.

2. Though the presence of monovalent cations is not an absolute requirement, we found that the optimal buffer contains 50–100 m*M* Na^+. K^+ ions would be more physiological; however, as the presence of K^+ ions influences the conformation of G-DNAs, they should be avoided if analysis of G4 quadruplex is carried out. Most protocols utilize C1 anions, but several alternatives are available. We favor acetate-based buffers for the RecQ helicases.

3. The ATP molecules are complexed with Mg^{2+} ions in the reaction buffer. The optimal ATP/Mg^{2+} ratio is generally around 1 (Harmon and Kowalczykowski, 2001). Most helicases will utilize 1 m*M* ATP efficiently, but this should be tested experimentally.

4. The configuration of Holliday junction substrates is Mg^{2+} dependent. Unwinding of these substrates might be inhibited at high Mg^{2+} concentrations.

5. DTT should be included in the buffer to maintain the reduced state of the enzyme molecules, therefore preventing aggregation and precipitation.

6. The presence of BSA (100 g/ml) in the reaction buffer is also protective.

In unwinding assays using a number of DNA helicases (BLM, WRN, UvrD, dmRecQ5) we found optimal rates of unwinding using the following reaction buffer (1):

66 m*M* Na-acetate
33 m*M* Tris-acetate, pH 7.8
1 m*M* ATP
1 m*M* Mg-acetate
100 g/ml BSA
1 m*M* DTT.

A 10 concentrated buffer (excluding ATP and Mg-acetate) can be prepared in advance.

Substrate Enzyme Ratio, Enzyme Concentration: Many laboratories studying the biochemistry of helicases find that the concentration of enzyme needed for detectable unwinding exceeds the substrate concentration; in some cases by many fold. This is a phenomenon that might be explained in several ways. It is possible that due to suboptimal reaction circumstances only a fraction of the protein is active enzymatically. The enzyme might also have lost full activity during purification. Several helicases have been shown to be oligomeric in solution (Patel and Picha, 2000, and see references therein); the oligomeric state of the enzyme might decrease the effective enzyme concentration and specific activity. BLM (Cheok *et al.*, 2005; Machwe *et al.*, 2005), WRN (Machwe *et al.*, 2005),

RECQL1 (Sharma *et al.*, 2005) and RECQ5 (Garcia *et al.*, 2004) have been shown to possess DNA strand annealing activity, which works against the unwinding activity. It is also possible that several helicase molecules, or possibly helicase oligomers, simultaneously engage on one DNA strand, like beads on a thread.

No direct experimental data support any single explanation of the above phenomenon. Most likely, several features of helicase action all contribute to a reaction kinetic that must be considered as a single turnover reaction.

Helicase Reaction

For detailed enzymatic analysis of helicases the reaction circumstances (i.e., optimal substrate and enzyme concentrations) should be determined experimentally. Following the substrate preparation and purification guidelines detailed previously, the concentration of the purified substrate will generally be in the low n*M* range (0.5–5 n*M*). The radioactive detection method enables one to use as low as a 0.5 fmol of the substrate. Using a much higher amount of substrate might be disadvantageous as the high substrate concentration can inhibit unwinding.

The optimal enzyme concentration should be determined by titrating the enzyme preparation by serial dilutions. The dilution must be carried out in the same dilution buffer that the enzyme was dissolved in originally, to avoid changing any other component (such as monovalent cation concentration) that might influence unwinding activity. An example for such a titration experiment can be seen in Fig. 2.

Procedure 1

1. Keeping everything on ice, prepare a serial dilution of the enzyme such that 1 l will contain the desired amount.
2. In a screw-capped microcentrifuge tube on ice, make up the required amount of master reaction mix as below:

   1 l 10 helicase buffer (see composition above)
   0.1 l 100 m*M* ATP
   1-x l 10 m*M* Mg-acetate (the substrate already contains 10 m*M* Mg^{2+})
   x l substrate (0.5–1 fmol)
   y l ddH_2O to make the final volume 9 l.

3. Mix well and dispense into screw-capped microcentrifuge tubes on ice, 9 l each.
4. Place in a 37 water bath or other incubation device.

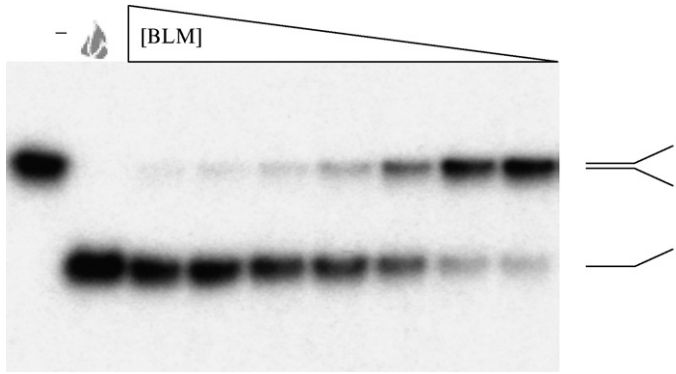

FIG. 2. An example of the concentration-dependence of unwinding. 25, 18.75, 15, 12.5, 10.7, 9.4, 8.3, and 0 fmol BLM was used to unwind approximately 2 fmol replication fork substrate in a 10- l reaction volume for 45 min. '–' indicates untreated substrate; the flame symbol depicts heat-denatured substrate.

5. Start the helicase reaction by the addition of 1 l diluted enzyme.
6. At the end of the incubation time, add 1.2 l 10 loading dye (50% glycerol; 100 m*M* Tris-HCl, pH 7.5; 50 m*M* EDTA; 4% SDS; 0.1% bromo-phenol-blue; 0.1% xylene-cyanol), mix, and put the tubes back on ice until electrophoresis.

Procedure 2

A more quantitative picture of helicase activity can be obtained by following the progress of the unwinding reaction in a timecourse.

1. Prepare 9 reaction termination tubes on ice that each contain 1.2 l 10 loading dye (see above).
2. In a screw-capped microcentrifuge tube set up reaction mixture for 10 samples on ice:

   10 l 10 helicase buffer (see composition above)
   1 l 100 m*M* ATP
   10-x l 10 m*M* Mg-acetate
   x l substrate (0.5–1 fmol)
   y l ddH_2O to make the final volume 100 l minus the amount of enzyme.
3. Mix and put into a 37 hotblock.
4. Add 10 the amount of enzyme that corresponds to the optimal dilution determined above in Procedure 1. Mix briefly.
5. Take a 10- l sample; add it to the first pre-prepared reaction termination tube.

6. At 30 s, 1, 2, 3, 5, 7, 10, and 15 min take a further 10- l sample as above.
7. Keep the collected samples on ice until electrophoresis.

It is possible to run simultaneously more than one timecourse since the addition of the enzyme, mixing, and putting the sample back on the hot-block takes approximately 10 s. In this case, the very first sample (time-point 0) should be taken before the addition of the enzyme, and each sample manipulation should be staggered by 10 s.

The amount of reaction product often reaches a plateau in 5–7 min, and therefore taking samples up to a maximum of 15 min is usually sufficient. Because the progress curve changes more rapidly in the beginning of the reaction, as many samples as possible should be taken during the first 5 min; after that the initial period sampling can be less frequent. In some cases, it might be necessary to extend the reaction duration to longer than 15 min. In this case, taking more samples is more favorable than rearranging the schedule outlined previously.

An example of a timecourse experiment carried out as described previously is shown in Fig. 3.

Electrophoresis and Detection: The reaction product (i.e., the released single-stranded oligonucleotide) is separated from the intact substrate by polyacrylamide gel electrophoresis. For the analysis of oligonucleotide-based, ''traditional'' substrates (Holliday junction, replication fork, static D-loops, etc.) 10% polyacrylamide; for G4 substrates 15% polyacrylamide; for plasmid-based D-loops 4–20% gradient polyacrylamide TBE gels are recommended. The electrophoresis tank should be chilled during gel running to avoid heating the sample, which could cause ''spontaneous'' denaturation of the substrate. Using the gels recommended above, running the gel for 60 min at a constant current of 25 mA usually provides sufficient separation of substrate and product.

After electrophoresis, the gel should be transferred to a Whatman 3MM paper and dried. Detection and quantification is best carried out using phosphorimaging systems, such as a Molecular Dynamics Storm device, and ImageQuant software (Amersham Biosciences, UK).

Safety Issues

It is important to stress that the handling of radioisotopes is potentially hazardous, and precautions should be taken to avoid contamination of work areas and personnel. The quantities of ^{32}P used in the previous procedures is quite low, and therefore the operator can protect himself or herself quite easily by carrying out the manipulations behind a 1-cm thick

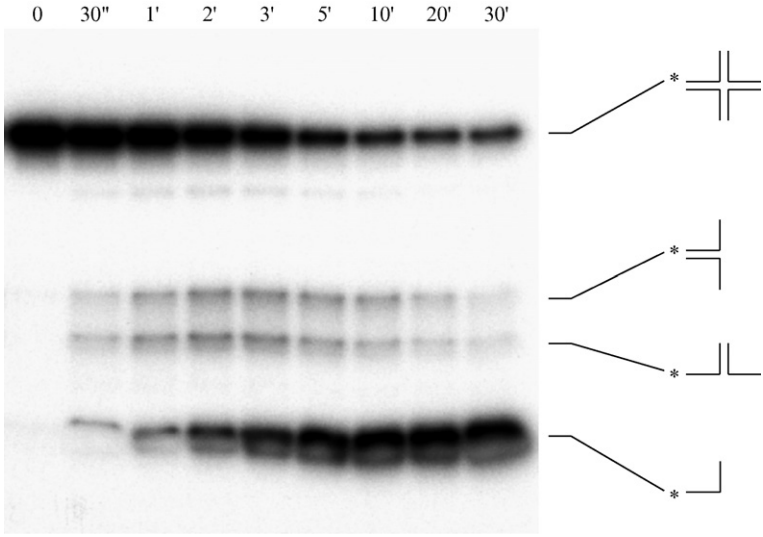

FIG. 3. An example of a timecourse experiment. The reaction contained 3.27 n*M* BLM and 56 p*M* Holliday junction substrate, and the reaction was monitored over a 30-min period. The side panel indicates the schematic structure of the products of unwinding.

Perspex screen of the type available from several commercial sources (e.g., Nalgene, Rochester, NY). It should also be noted that materials and equipment such as gel tanks inevitably become contaminated with low levels of ^{32}P. Safety rules vary from country to country and therefore it is important to consult with local radiation safety personnel to receive training and guidance in the safe handling of radioisotopes.

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