A novel DNA helicase, scHelI, has been purified from whole cell extracts of Saccharomyces cerevisiae using biochemical assays to monitor the fractionation. The enzyme unwinds partial duplex DNA substrates, as long as 349 base pairs in length, in a reaction that is dependent on either ATP or dATP hydrolysis. scHelI also catalyzes a single-stranded DNA-dependent ATP hydrolysis reaction; the apparent $K_m$ for ATP is 325 $\mu$M. The unwinding reaction on circular partial duplex substrates is biphasic, with a fast component occurring within 5 min of the initiation of the reaction and a slow component continuing to 60 min. This is in contrast to the ATP hydrolysis reaction, which exhibits linear kinetics for 60 min. The direction of the unwinding reaction is 5' to 3' with respect to the strand of DNA on which the enzyme is bound. The unwinding reaction is strongly stimulated by the addition of Escherichia coli single-stranded DNA-binding protein when long partial duplex substrates are used.

The enzymatic activity of scHelI copurifies with a polypeptide of 135 kDa as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The polypeptide sediments as a monomer in a glycerol gradient in the presence of 0.2 M NaCl.

DNA helicases are essential components of the machinery involved in the replication, recombination, and repair pathways of DNA metabolism in the cell. These enzymes disrupt the hydrogen bonds between base pairs of duplex DNA to generate transiently single-stranded regions. The mechanism by which this occurs is unknown but may involve translocation through duplex DNA in a reaction that is fueled by nucleoside 5'-triphosphate (NTP) hydrolysis. In Escherichia coli, and the bacteriophage that infect them, more than a dozen DNA helicases have been identified and characterized both biochemically and genetically (1). These analyses have identified the biochemical pathways in which these enzymes act and have allowed in vitro reconstitution of several pathways for a more detailed understanding of the enzymatic function of helicases and their interaction with other proteins.

Knowledge regarding eukaryotic helicases and their roles in cellular DNA metabolism is less complete. Only a few eukaryotic helicases have been characterized both biochemically and genetically. These include the simian virus 40 large T antigen (2), the herpes simplex virus helicase-primase (3), and three DNA helicases from the yeast Saccharomyces cerevisiae; Rad3 protein (4, 5), Pif1 protein (6, 7), and Srs2 protein (8, 9). The three yeast helicases are encoded by the cellular genome, although Pif1 is thought to function in the mitochondria (7). In each case, the gene was identified first by mutation (4, 6, 8) and subsequently, using biochemical assays, the gene product was shown to catalyze a helicase reaction (5, 7, 9). In addition to the helicases described above, several other eukaryotic DNA helicases have been identified by biochemical assay. A listing of these helicases has been presented elsewhere (10) and includes enzymes from calf thymus (11-14) and cultured human cells (15-17). None of these proteins have been characterized genetically. Even more numerous are the putative helicases that have been identified by molecular genetic techniques. In each case, sequence homology suggests that the gene may encode a helicase, but gene products have not yet been shown to have helicase activity by biochemical assay (18-21). It is apparent that both genetic and biochemical information are required to identify a precise role for these proteins in nucleic acid metabolism.

The yeast S. cerevisiae has been well characterized genetically, is highly amenable to molecular genetic analysis, and can be used for the biochemical purification of enzymes. For this reason, we have chosen yeast as a eukaryotic model system in which to identify and characterize individual DNA helicases both biochemically and genetically. This communication reports the purification and initial biochemical characterization of a new DNA helicase from yeast.

**EXPERIMENTAL PROCEDURES**

**Materials**

- **Yeast Cells**—A protease-deficient strain of S. cerevisiae, CB001 (MATa leu2 trp1 ura3 prb pep4::URA3), was used for the purification. Cultures (200 liters) were grown at 30°C in YPD media (1% yeast extract/2% bacto-peptone/2% dextrose) in a New Brunswick Scientific Fermatron 250-liter fermentor to a density of approximately $5 \times 10^7$ cells/ml. Cells were harvested by centrifugation and stored at −70°C until lysed. The yield was approximately 2.5 kg of cells (wet weight)/fermentor.

- **DNA and Nucleotides**—Bacteriophage M13 mp7 replicative form I (RF I) and ssDNA were prepared as previously described (22). SupercHonel DNA (pBSoriT) was the pBluescript vector (Stratagene) containing a cloned insert as described elsewhere (23). Linear double-stranded DNA was pBSoriT cut with XmnI. Unlabeled nucleotides were from U.S. Biochemical Corp. [α-32P]CTP and [2,8-3H]ATP were from Amersham Corp.

- **Enzymes**—Restriction endonucleases and DNA polymerase I (large fragment) were from New England Biolabs or U.S. Biochemical. The reaction conditions used were those suggested by the supplier. Proteinase K was from Boehringer Mannheim. E. coli helicase II was 1

1. The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; scHelI, Saccharomyces cerevisiae helicase I; SSB, Escherichia coli single-stranded DNA binding protein; bp, base pair(s); ATP-S, adenosine 5'-O-(thiotriphosphate); HPLC, high pressure liquid chromatography.
purified as previously described (24). E. coli helicase I, purified as previously described (25), was the generous gift of B. Morton (this lab). E. coli SSB was purified from an overproducing strain of E. coli.

Both the E. coli strain and the purification procedure were kindly provided by Dr. R. McMacken (Johns Hopkins University, Baltimore).

Other Materials—Glass beads (0.5 mm), heparin-agarose, and ATP-agarse, linked through ribose hydrobodies, were from Sigma. Double-strand DNA-cellulose (12.6 mg of DNA/g of cellulose) was from U. S. Biochemicals. Poly(ethylenimine) (Polymin-P) was from Aldrich and phoshocellulose (P-cell) was from Whatman. Anion exchange high pressure liquid chromatography (HPLC) was performed on a Rainin system using a Hypersil A column (1.0 × 10.0 cm) from Rainin.

Buffers—Lysis buffer was 200 mM Tris-HCl (pH 8.0), 8 mM EDTA, 6 mM 2-mercaptoethanol, 10% glycerol, and 0.1% Brij-35. Buffer A was 400 mM KPO, (pH 7.0), 5 mM 2-mercaptoethanol, 1 mM EDTA, 100 mM KC1, and 10% glycerol. Buffer B was 20 mM Tris-HCl (pH 7.5), 5 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.1 M NaCl, and 10% glycerol. Buffer C was 20 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 0.1 mM EDTA, 0.1 M NaCl, 0.01% Nonidet P-40, and 15% glycerol. Buffer D was 20 mM Tris-HCl (pH 8.0), 5 mM dithiothreitol, 0.1 mM EDTA, 50 mM NaCl, 0.01% Nonidet P-40, and 15% glycerol. Buffer E was 20 mM KPO, (pH 7.0), 5 mM dithiothreitol, 0.1 mM EDTA, 0.1 M NaCl, 0.01% Nonidet P-40, and 15% glycerol. Storage buffer was 0.1 M NaCl, 0.01% Nonidet P-40, and 15% glycerol. ATPase activity was measured at 30°C in the presence of sodium dodecyl sulfate was by the method of Laemmli (21784).

Methods

DNA Helicase Substrates—The helicase substrates used in this study were either circular or linear partial duplex DNA molecules as previously described (24, 26). Circular partial duplex substrates were constructed by annealing the complementary strand of a specific M13 mp7 RF1 HaeIII restriction fragment to M13 mp7 ssDNA and labeling the 3' terminus using [a-32P]dCTP and DNA polymerase I (large fragment) previously described (24).

Helicase Activity Assay—The helicase activity assay measures the displacement of a [32P]DNA fragment from a partial duplex DNA substrate. Partial duplex DNA (20 μg) was eluted from an HPLC column (1.2 cm) that had been equilibrated in buffer E. The column was washed with 5 volumes of buffer C containing 0.2 M NaCl and 10% glycerol and developed with a linear 15-column volume gradient from 0.2 to 0.7 M NaCl in buffer E. Fractions containing peak ATPase activity eluting between 0.47 and 0.57 M NaCl were pooled (fraction V) and dialyzed against buffer D until the conductivity was equivalent to that of buffer D. After dialysis, fraction V was loaded onto a 0.5-ml heparin-agarose column (0.8 × 1.0 cm) from Rainin and eluted with a linear 10-column volume gradient from 0.2 to 0.8 M NaCl. Fractions containing the second peak of activity were pooled and dialyzed against buffer F (fraction IV).

Fraction IV was loaded onto a 4-ml heparin-agarose column (1.0 × 4.5 cm) equilibrated with buffer C. The column was washed with 5 volumes of buffer C containing 0.2 M NaCl and developed with a linear 15-column volume gradient from 0.2 to 0.7 M NaCl in buffer E. Fractions containing peak ATPase activity eluting between 0.47 and 0.57 M NaCl were pooled (fraction V) and dialyzed against buffer D until the conductivity was equivalent to that of buffer D. After dialysis, fraction V was loaded onto a 0.5-ml heparin-agarose column (0.8 × 1.0 cm) that had been equilibrated in buffer E. The column was washed with 5 volumes of buffer E containing 0.2 M NaCl and eluted with a 15-column volume gradient from 0.2 to 0.5 M NaCl in buffer E. Peak ATPase activity eluted at 0.27–0.35 M NaCl, and fractions containing the activity were pooled (fraction VII) and dialyzed against buffer C (fraction VIII). Fraction VII was loaded onto a 0.5-ml heparin-agarose column (0.8 × 1.0 cm) equilibrated in buffer C containing 0.2 M NaCl and 10% glycerol. The column was washed with 5 volumes of buffer C containing 0.4 M NaCl and 10% glycerol and developed using a 15-column volume linear gradient from 0.2 to 0.5 M NaCl in buffer C. Fractions containing peak ATPase activity were pooled and dialyzed against buffer C (fraction IX). ATPase activity was measured at 30°C in the presence of ssDNA, and fractions containing a peak of ATPase activity eluting between 0.52 and 0.60 M NaCl were pooled (fraction X) and dialyzed against buffer C (fraction XI).

RESULTS

PURIFICATION OF DNA Helicase I (scHel) FROM Yeast—The purification of S. cerevisiae DNA helicase I (scHel) is summarized in Table I. Whole cell lysates were initially fractionated using polymin-P (0.2% final concentration) to precipitate nucleic acids and associated proteins. It has been reported that Rep protein from E. coli sediments with the nucleic acid

RESULTS

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pellet in polymin-P precipitations (30). For this reason, the polynuc-P precipitate was dialyzed and chromatographed on phosphocellulose as described under "Experimental Procedures." This step resolved three peaks of ATPase activity (Fig. 1). Further fractionation of the second and third peaks of DNA-dependent ATPase activity revealed three distinct DNA helicases (data not shown). The second peak contains scHeIl, and the third peak has a mixture of two DNA helicases, scHeII and scHeIl. In subsequent purification steps, both ATPase and helicase activity were monitored. However, contaminating nuclease activity prevented quantitation of helicase activity until fraction VIII, the second heparin-agarose pool.

The DNA-dependent ATPase activity eluting from the phosphocellulose column at approximately 0.6 M KCl was further fractionated on dsDNA-cellulose, which resolved two peaks of DNA-dependent ATPase activity (data not shown). The first activity peak, which eluted at 0.4 M NaCl, had DNA helicase activity but was heavily contaminated with nuclease activity at this stage of the purification. This activity has been further purified and characterized as scHeIl. The ssDNA-dependent ATPase eluting at 0.47-0.60 M NaCl had DNA helicase activity and appeared as a single broad peak. Further fractionation on heparin-agarose, an anion exchange matrix, and ATP-agarose resolved the DNA helicase activity from most of the contaminating nuclease activity but increased the ATPase specific activity 3-fold (Table I). After fractionation on a second heparin-agarose column, aliquots from each fraction were resolved on a polyacrylamide gel run in the presence of sodium dodecyl sulfate. Both helicase and ssDNA-dependent ATPase activity cochromatographed with a polypeptide of approximately 135 kDa, but high levels of a low molecular weight polypeptide were present in fractions containing peak enzymatic activity (data not shown). Preparative glycerol gradient ultracentrifugation resolved the 135-kDa polypeptide from the low molecular weight polypeptide (Fig. 2) and from several minor contaminants between 60 and 100 kDa. Both helicase and ATPase activity cosedimented with the 135-kDa polypeptide, which was the predominant polypeptide present in peak activity fractions (Fig. 2, lower panel, fractions 9-11). Nuclease activity, measured as degradation of the helicase substrate, was not detected in the gradient. The helicase/ATPase activity sedimented between the 7.4 s and the 4.3 s markers consistent with a monomeric protein with a relative molecular mass of approximately 135,000. We conclude that scHeIl is a monomeric protein with an $M_r$ of 135,000.

**Reaction Requirements**—Purified scHeIl unwound the double-stranded region of a 71-bp partial duplex molecule in a reaction that required ATP or dATP and MgCl$_2$ or MnCl$_2$ (Table II). None of the remaining NTPs (dNTPs) could effectively substitute for ATP when present at a concentration of 1.0 mM. In the absence of MgCl$_2$, or in the presence of 10 mM EDTA, helicase activity was below detectable levels. DNA helicase activity was dependent on MgCl$_2$ with an optimum concentration between 1.0 and 2.0 mM (Fig. 3B) in the presence of 2.0 mM ATP. Titrations using MnCl$_2$, ZnCl$_2$, or CaCl$_2$, substituted for MgCl$_2$, indicated that MnCl$_2$ could substitute for MgCl$_2$ but was less effective (data not shown). ZnCl$_2$ and CaCl$_2$ could not substitute for MgCl$_2$ in the helicase reaction. The poorly hydrolyzed ATP analog, ATP$_7$S, could not substitute for ATP in the unwinding reaction, suggesting a requirement for concomitant NTP hydrolysis. ATP$_7$S has also been shown to be a competitive inhibitor of the unwinding reaction (data not shown). NaCl concentrations greater than 40 mM inhibited the unwinding reaction (Fig. 3C), and, at 200 mM NaCl, the unwinding reaction was diminished by more than 95% (Fig. 3C and Table II).

The pH optimum for the unwinding reaction ranged broadly, from 6.3 to 8.0 (Fig. 3A). Unwinding dramatically decreased below pH 6.5 and was barely detectable at pH 5.5. The activity above pH 8.0 was only slightly diminished. Three different buffers were used to span the pH range, and no significant buffer effects were measured at the points of overlap (pH 6.5 and 7.5).

**The Unwinding Reaction Catalyzed by scHeIl Is Dependent on Protein Concentration**—scHeIl catalyzed the displacement of DNA fragments of various lengths that had been annealed

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**Table I**

| Fraction | Total Protein (mg) | Total ATPase Activity (units) | Specific activity (units/mg) |
|----------|-------------------|------------------------------|----------------------------|
| Fraction I | 59.400 | ND$^a$ | ND |
| Fraction II | 5.900 | ND | ND |
| Fraction III | 99 | 35,040 | 353 |
| Fraction IV | 4.79 | 12,564 | 2,623 |
| Fraction V | 1.35 | 6,528 | 4,835 |
| Fraction VI | 1.29 | 9,456 | 7,330 |
| Fraction VII | 0.220 | 3,174 | 14,429 |
| Fraction VIII | 0.145 | 1,753 | 12,089 |
| Fraction IX | 0.012 | 1,658 | 138,200 |

$^a$ ND, not determined.

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$^2$ D. W. Bean and S. W. Matson, manuscript in preparation.
A DNA Helicase from S. cerevisiae

**FIG. 1.** Phosphocellulose chromatography of fraction II. Fraction II was dialyzed, divided into four aliquots (each representing 450 g of cells), and chromatographed on phosphocellulose as described under "Experimental Procedures." The ssDNA-dependent ATPase activity (C) was measured as described. The elution positions of *S. cerevisiae* DNA helicases I, II, and III (scHelI, II, III) have been indicated.

**FIG. 2.** DNA unwinding and DNA-dependent ATPase activities cosediment with a 135-kDa polypeptide. Fraction VIII was sedimented on a continuous glycerol gradient as described under "Experimental Procedures." *Upper panel,* both ssDNA-dependent ATPase and helicase activity were measured as described under "Experimental Procedures." Hydrolysis of ATP was measured in a 10-min reaction that contained 0.2 μl of the indicated fraction. Displacement of a 71-nucleotide DNA fragment from a partial duplex substrate, using 0.1 μl of the indicated fraction, is indicated. The sedimentation markers were aldolase (7.4 s) and bovine serum albumin (4.3 s). *Lower panel,* aliquots (100 μl) of indicated gradient fractions were resolved on an 8.0% polyacrylamide gel run in the presence of sodium dodecyl sulfate. Proteins were visualized by staining with Coomassie Blue. The marker proteins were as follows: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa).
to M13 mp7 ssDNA (Figs. 4 and 6). The unwinding reaction was proportional to protein concentration on substrates with either 71- or 216-bp partial duplex regions (Fig. 4). As the length of the duplex region increased, the fraction of the substrate unwound by scHelI decreased. Unwinding of a 343-bp partial duplex substrate was barely detectable (see Fig. 6). 

E. coli SSB Stimulates scHelI—The 71-bp partial duplex substrate was efficiently unwound by scHelI, yet long duplex regions were found to be poorly unwound. For this reason, the effect of E. coli SSB on the unwinding reaction catalyzed by scHelI was tested using a 71-bp partial duplex substrate. The addition of up to 66 nM (tetramer) E. coli SSB increased the fraction of the 216-nucleotide fragment displaced from 5% to nearly 75% of the total substrate, a 15-fold stimulation (Fig. 5). The concentration of SSB required to saturate the ssDNA is 35 nM (tetramer), assuming a DNA concentration of 2 μM, and, under the solution conditions of the unwinding reaction, an SSB binding site size of 56 nucleotides (31). Control experiments demonstrated that SSB alone was not able to promote the unwinding reaction (data not shown). Thus, the addition of a single-stranded DNA-binding protein increased the unwinding of longer duplex regions catalyzed by scHelI. Stimulation of the unwinding reaction decreased at high SSB concentrations (greater than 66 nM tetramer).

The Unwinding Reaction Catalyzed by scHelI Is Time-dependent—The kinetics of the unwinding reaction catalyzed by scHelI exhibited both a fast and a slow component (Fig. 6). During the first 5 min, the rate of unwinding was rapid with both the 71-bp substrate and the 343-bp partial duplex substrate plus E. coli SSB. Unwinding of the 343-bp substrate, in the presence of SSB, continued at a linear, but much slower, rate out to 60 min. Further unwinding of the 71-bp partial duplex substrate could not be detected after 20 min. Without SSB, the unwinding of the 343-bp partial duplex substrate was barely detectable, even after a 60-min incubation. The kinetics of unwinding were the same whether the reactions were initiated by the addition of enzyme or by the addition of ATP (data not shown).

ScHelI unwinds longer duplex regions less effectively than short duplex regions (Figs. 4 and 6), which has also been reported for a number of prokaryotic helicases (26, 32). However, the addition of SSB increased the fraction of the 343-bp partial duplex substrate unwound to that observed using the 71-bp partial duplex substrate. The addition of SSB served to stimulate the unwinding of a 71-bp partial duplex substrate only modestly (data not shown).

The ATPase Activity of scHelI Is ssDNA-dependent—As expected, scHelI also catalyzed the hydrolysis of ATP in a reaction that was ssDNA-dependent (Fig. 7). In the absence of ssDNA, ATP hydrolysis was barely detectable. Both M13 mp7 ssDNA and linear poly(dT) (approximately 1100 nucleotides in length) served as effectors in the ATP hydrolysis reaction. Interestingly, the rate of ATP hydrolysis in the presence of poly(dT) was nearly twice that measured in the presence of M13 mp7 ssDNA. Supercoiled dsDNA, linear dsDNA, and poly(A) supported a low level of ATP hydrolysis. The titration curves presented in Fig. 7 indicate a Kₐₐ value for these three nucleic acids at least 20-fold higher than that of ssDNA, where Kₐₐ is defined as the nucleic acid concentration required to achieve ATP hydrolysis at one-half the maximum velocity, at saturating concentrations of ATP. Poly(C) and tRNA (data not shown) failed to support the ATPase reaction. In reactions containing 2 μM M13 mp7 ssDNA, the addition of 13 nM E. coli SSB (tetramer), sufficient to achieve about 35% saturation of the ssDNA, had little or no effect on the ATPase activity of scHelI. The addition of 130 nM SSB reduced ATPase activity by 30% (data not shown). This concentration of SSB is more than three times that required to fully coat the ssDNA. The apparent Kₐₐ for rATP was determined to be 325 μM under standard reaction conditions (data not shown).

The DNA-dependent ATPase activity catalyzed by scHelI,

TABLE II

| Reaction conditions for scHelI |
|-----------------------------|
| DNA helicase assays were performed as described under “Experimental Procedures” using a 71-bp partial duplex substrate and 0.025 units of scHel ATPase activity/reaction. Reaction mixtures were incubated for 20 min. The values reported are the mean ± S.D. of the ratios obtained from three independent determinations. |
| Reaction mixture | Helicase activity* |
|------------------|--------------------|
| Complete (1.0 mM ATP) | 100.0 |
| -ATP | 2.1 ± 2.9 |
| +1.0 mM GTP | 5.8 ± 5.4 |
| +1.0 mM CTP | 2.0 ± 0.6 |
| +1.0 mM UTP | 5.9 ± 3.3 |
| +1.0 mM dATP | 100.7 ± 8.6 |
| +1.0 mM dGTP | 6.1 ± 5.2 |
| +1.0 mM dCTP | 3.0 ± 1.5 |
| +1.0 mM dTTP | 1.8 ± 1.8 |
| +0.2 mM ATP₇S | 1.0 ± 1.7 |
| +MgCl₂ | 3.4 ± 4.0 |
| -MgCl₂ | 2.2 ± 3.2 |
| +200 mM NaCl | 2.9 ± 2.5 |
| +4 mM MnCl₂ | 45.8 ± 4.6 |
| +4 mM ZnCl₂ | 15.9 ± 4.0 |
| +4 mM CaCl₂ | 1.6 ± 1.3 |

* Unwinding values are expressed relative to unwinding in the presence of 1.0 mM ATP under standard reaction conditions. Under these conditions, approximately 50% of the partial duplex substrate was unwound.

![Fig. 3. Requirements of the scHel DNA unwinding reaction. Helicase reactions were performed as described under “Experimental Procedures” using 0.025 ATPase activity units of scHel (panels A and B) or 0.012 ATPase activity units of scHel (panel C) and a 71-bp partial duplex substrate in 20-min reactions. The data reported represent the average of at least three determinations. Panel A, helicase reactions were performed in three different buffer systems, as described under “Experimental Procedures,” with overlapping pH values at 6.5 and 7.5. Since no buffer effects were noted, all values were averaged. Panel B, helicase reactions were performed as described under “Materials and Methods,” supplemented to the indicated MgCl₂ concentration. Panel C, helicase reaction mixtures were as described under “Materials and Methods,” supplemented to the indicated NaCl concentration.](image-url)
in the presence of ssM13 mp7, displayed linear kinetics throughout a 60-min incubation (Fig. 8). This is in contrast to the biphasic reaction kinetics observed when the DNA unwinding reaction was monitored.

**scHelI Unwinds Duplex DNA in a 5′ to 3′ Direction**—To determine the polarity of the unwinding reaction catalyzed by scHelI, the DNA substrate shown in Fig. 9A was constructed. This molecule contains a 143-bp duplex region at the 5′-end, a 202-bp region at the 3′-end, and a long internal region of ssDNA to which the helicase may initially bind. An enzyme that unwinds duplex DNA with a 5′ to 3′ polarity with respect to the ssDNA on which it initially binds is expected to displace the 202-nucleotide DNA fragment; an enzyme with the opposite polarity will unwind the 143-nucleotide DNA fragment.

scHelI, in the presence of *E. coli* SSB (130 nM, tetramer), catalyzed the unwinding of the 202-nucleotide DNA fragment with no detectable unwinding of the 143-nucleotide DNA fragment (Fig. 9B, lane 4) indicating a 5′ to 3′ polarity for the unwinding reaction. ScHelI without added SSB was not capable of efficiently displacing the partial duplex direction substrate (Fig. 9B, lane 5). By increasing the amount of scHelI 10-fold, unwinding of the 202-nucleotide fragment could be detected without SSB (data not shown). Thus, the addition of SSB did not alter the polarity of unwinding but served to stimulate the unwinding reaction. Control experiments were performed using *E. coli* DNA helicase II (Fig. 9B, lane 3), a 3′ to 5′ helicase; *E. coli* DNA helicase I (Fig. 9B, lane 2), a 5′ to 3′ helicase; and SSB in the absence of a helicase, which was incapable of displacing either DNA fragment (Fig. 9B, lane 1). We conclude that scHelI catalyzes a 5′ to 3′ unwinding reaction.

**DISCUSSION**

We have used a direct, *in vitro* helicase assay to identify, purify, and characterize a previously undescribed ATP-dependent DNA helicase activity from whole cell lysates of *S. cerevisiae*. The purified enzyme consists of a single polypeptide with a molecular mass of 135 kDa as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The sedimentation velocity of the enzyme in a continuous glycerol gradient suggests the protein exists as a monomer in solution. This enzyme has been designated scHelI. scHelI unwinds duplex DNA in a reaction that depends on the addition of both MgCl₂ and ATP (or dATP). The poorly hydrolyzed ATP analog, ATPγS, could not substitute for ATP in the unwinding reaction, indicating a requirement for concomitant ATP hydrolysis as has been observed for helicase enzymes isolated from other sources (1). Unwinding is in the 5′ to 3′ direction with respect to the strand of DNA on which the enzyme initially binds.

Kinetic studies indicate the unwinding reaction catalyzed by scHelI consists of a rapid phase lasting approximately 5 min, during which most of the unwinding occurs, followed by a slow phase in which duplex DNA is steadily displaced for at least 60 min. The fraction of substrate molecules unwound during the rapid phase is proportional to the ratio of substrate to enzyme. When the concentration of substrate is decreased by one-half, the fraction of unwound molecules is nearly doubled. This is in contrast to the kinetics of ATP hydrolysis, which are linear for at least 60 min over a range of DNA effector concentrations, ATP concentrations, and enzyme concentrations. These observations suggest a rapid associa-
FIG. 6. Kinetics of unwinding partial duplex substrates of different lengths. Unwinding reactions were performed as described under “Experimental Procedures” with the following modifications. Reaction volumes were increased to 180 µl, and reactions were initiated by the addition of 0.135 ATPase activity units of scHelI. Aliquots (20 µl) were withdrawn at the indicated times and stopped by the addition of EDTA and loading dyes as described under “Experimental Procedures.” Reaction mixtures contained partial duplex substrates of 71 bp (Δ), 343 bp (○), or 343 bp plus 33 nM SSB (tetramer) (●). Each data point represents the mean ± S.D. of at least three determinations.

Fig. 7. The ATPase activity is dependent on ssDNA. ATPase reactions were performed as described under “Experimental Procedures” using 0.5 ATPase activity units of scHelI, 2.1 mM ATP, and increasing concentrations of poly(dT) (△), M13 mp7 ssDNA (○), supercoiled plasmid DNA (●), linear dsDNA (●), poly(A) (□), or poly(C) (◇). Each data point is the mean ± S.D. of at least three determinations.

Fig. 8. Kinetics of ATP hydrolysis catalyzed by scHelI. ATPase reactions were performed as described under “Experimental Procedures” with the following modifications. The reaction mixture volume was increased to 50 µl and contained 2.1 mM [3H]ATP. Reactions were initiated by the addition of 0.25 ATPase activity units of scHelI, and 5 µl aliquots of the reaction were withdrawn at the indicated times for determination of [3H]ADP production. The data represent the mean ± S.D. of three determinations.

...tion of the enzyme with the partial duplex substrate followed by unwinding of the duplex region and a continued hydrolysis of ATP, while the enzyme remains associated with the ssDNA circle. Dissociation and association with other partial duplex substrate molecules is apparently slow, resulting in the slow phase of unwinding kinetics. Unwinding of other substrates, such as linear partial duplex DNA, fully duplex linear DNA, and DNA:RNA hybrid substrates, must be tested to further characterize the unwinding reaction.

The purification of scHelI was monitored using a partial duplex substrate with 71 bp of duplex DNA. When the length of the duplex region on the partial duplex substrate was increased, the fraction of the substrate unwound by scHelI decreased. In fact, unwinding could barely be detected when the duplex region was 343 bp in length (Fig. 6). However, the unwinding reaction measured using longer partial duplex substrates was greatly stimulated by the addition of E. coli SSB (Figs. 5 and 6). The extent of unwinding of the 343-bp partial duplex substrate in the presence of E. coli SSB was increased to approximately that measured using the 71-bp duplex substrate. In addition, only slight stimulation of unwinding was achieved by incubation of the 71-bp partial duplex substrate with SSB. The stimulatory effects of SSB are likely to have an impact on a step or steps in the unwinding reaction that become more important as the length of the duplex region is increased. Since SSB is not able to catalyze the unwinding of duplex DNA, it seems likely that SSB functions by coating the ssDNA present in the reaction to enhance unwinding. We presume that SSB is bound to both the ssDNA along which the enzyme translocates to move to the duplex region and the ssDNA fragment produced during the course of the unwinding reaction. Interestingly, the addition of SSB has little effect...
on the ATP hydrolysis reaction catalyzed by scHelI. This is in contrast to what has been observed for the majority of prokaryotic helicases that have been described (1). The stimulatory effect of SSB on the unwinding reaction catalyzed by scHelI is similar to that previously reported for a minority of the prokaryotic enzymes. For example, the unwinding reaction catalyzed by DnaB protein from E. coli is stimulated 6-fold by the addition of SSB (33), while PriA protein (34) shows nearly absolute dependence on SSB for unwinding of long duplex regions. Among helicases identified from eukaryotes, several of the calf thymus helicases are stimulated by E. coli SSB or the eukaryotic SSB analogs (11, 12), while a DNA helicase isolated from a human cell line is dependent on eukaryotic SSB for unwinding activity (17).

Five DNA helicases, isolated from yeast, have been described biochemically as follows: Rad3 protein (5), ATPase III (35), Pif1 protein (7), Srs2 protein (9), and an RF-C-associated helicase (36). scHelI appears to be distinct from each of these previously described helicases on the basis of biochemical and/or physical criteria. The molecular masses of Rad3 (89 kDa), Pif1 (97 kDa) and Srs2 (134 kDa) have been deduced from DNA sequence data and confirmed for the purified proteins. ATPase III (63 kDa) and RF-C-associated helicase (60 kDa) have been characterized on polyacrylamide gels run in the presence of sodium dodecyl sulfate. Only the SRS2 gene product is large enough to be scHelI; however, biochemical criteria distinguish the two enzymes. The Srs2 protein has been shown to display a 3' to 5' polarity of unwinding (9) using a partial duplex substrate similar to the one used in this study (Fig. 9). ScHelI, in contrast, exhibits a 5' to 3' polarity in the unwinding reaction.

A number of genes in S. cerevisiae have been shown to share sequences that are found in DNA helicases. Several of these genes, including FUN30 (18), STH1 (19), and RAD5 (20) are of approximately the correct size to encode scHelI. Of these, only rad5 mutants have thus far been shown to display phenotypes consistent with a helicase function. The RAD5 gene has been placed in the RAD6 epistasis group of DNA repair proteins, and it encodes a polypeptide of 134 kDa (20). Thus, Rad5 protein and scHelI could be equivalent, but, since the gene product of RAD5 has not been characterized biochemically and the gene encoding scHelI has not yet been cloned, we can only speculate on this relationship.

We conclude, on the basis of both polypeptide size and biochemical properties, that scHelI is a previously undescribed helicase. Any speculation as to the role played by this protein in nucleic acid metabolism must await cloning of the gene encoding this protein and a description of the phenotype of scHelI mutants.

Acknowledgments—We thank Susan Whitfield for preparation of the artwork.

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