A Single-Stranded DNA Exonuclease from *Schizosaccharomyces pombe*†

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ABSTRACT: We have purified to near homogeneity a DNA exonuclease from meiotic cells of *Schizosaccharomyces pombe*. The enzyme, designated exonuclease II (ExoII), had an apparent molecular weight of 134 000 and was abundant in the cell. It specifically degraded single-stranded DNA in the 5′→3′ direction with an apparent K_m for 5′ DNA ends of 3.6×10^{-11} M and produced 5′ deoxynucleoside monophosphates. Its mode of degradation is similar to that of the RecJ protein from *Escherichia coli*; ExoII may, therefore, be involved in genetic recombination and DNA damage repair.

Exonucleases play an important role during genetic recombination and the repair of DNA damage. Several recombination models [reviewed in Orr-Weaver and Szostak (1985)] propose the resection of one strand from duplex DNA to allow the annealing of the remaining strand with a complementary strand from a second DNA molecule. Enzymes degrading a DNA strand in the 5′→3′ direction have been especially implicated in such functions, possibly reflecting the polarity required by strand annealing activities.

λ exonuclease, exonuclease VIII from *Escherichia coli*, and T7 gene *6* exonuclease degrade ds DNA[1] in the 5′→3′ direction (Little, 1967; Joseph & Kolodner, 1983; Kerr & Sadowski, 1972) and are required for recombination in *E. coli* and its bacteriophages (Gillen et al., 1981; Stahl, 1986; Kerr & Sadowski, 1975). RecJ protein from *E. coli* is the only known DNA exonuclease that specifically degrades ss DNA in the 5′→3′ direction (Lovett & Kolodner, 1989). Its activity is required for recombination by the *recF* pathway (Lovett & Clark, 1984) and for recombination and UV resistance in *recD* mutants (Lovett et al., 1988; Lloyd et al., 1988).

To test the possibility that such nucleases are required for recombination and repair in eukaryotes, we have begun characterization of exonucleases in the yeast *Schizosaccharomyces pombe*. We have recently reported a meiotically induced 5′→3′ ds DNA exonuclease, exonuclease I (ExoI), from *S. pombe* (Szankasi & Smith, 1992). In this paper, we describe another exonuclease from *S. pombe*, exonuclease II (ExoII). This enzyme was present in both meiotic and mitotic cells and specifically degraded ss DNA in the 5′→3′ direction. Its similarity with RecJ exonuclease from *E. coli* suggests that ExoII is involved in recombination or DNA damage repair in *S. pombe*.

MATERIALS AND METHODS

Enzymes and Chemicals. Restriction endonucleases, *E. coli* DNA polymerase I large fragment, T4 polynucleotide kinase, and calf intestinal phosphatase were obtained from New England Biolabs, Boehringer Mannheim, or U.S. Biochemicals and used according to the manufacturer's recommendations. Unless indicated differently, all other reagents were purchased from Sigma, J. T. Baker, and U.S. Biochemicals. Yeast RNA was obtained from BDH Biochemicals (Poole, England).

Nucleic Acids. ^{3}H-Labeled T7 DNA was prepared as described earlier (Szankasi & Smith, 1992) and had a specific activity of $(5–8) \times 10^4$ cpm/nmol of nucleotide. [^{3}H]T7 DNA was dephosphorylated and labeled with ^{32}P at the 5′ end as described (Sambrook et al., 1989) except that solutions were carefully mixed by inversion of the tube, avoiding shearing of the DNA. Unlabeled M13mp18 RFI DNA was prepared as described (Szankasi & Smith, 1992), cleaved with the restriction endonuclease *Sau*3A, and labeled at either the 3′ end using [α-^{32}P]dCTP or the 5′ end using [γ-^{32}P]ATP (Sambrook et al., 1989) to 10^7 cpm/μg of DNA. Internally ^{32}P-labeled DNA was prepared as follows: 1.6 pmol of M13 sequencing primer (–47, New England Biolabs) was 5′ phosphorylated (Sambrook et al., 1989) and mixed with 2 μg of M13mp18 virion DNA in 20 μL of 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT. The solution was heated to 65 °C for 5 min and slowly cooled to room temperature. The complementary strand was synthesized with 2.5 units *E. coli* DNA polymerase I large fragment for 1 h at room temperature, in the presence of 10 μCi of [α-^{32}P]-dCTP or [α-^{32}P]TTP (diluted to 80 Ci/mmol; NEN, Boston, MA) and 500 pmol of each of the three unlabeled dNTPs. The DNA was extracted with phenol and chloroform and precipitated twice with ethanol.

Thin-Layer Chromatography. Reaction products were separated on a polyethylenimine–cellulose plate with fluorescent indicator (J.T. Baker Chemical Co.) essentially according to Randerath and Randerath (1967) as described (Szankasi & Smith, 1992). A total of 20 nmol of unlabeled standards and 1/50 of the exonuclease reactions with internally ^{32}P-labeled DNA were loaded on the chromatogram. Unlabeled standards were visualized under UV light. Labeled reaction products were visualized by autoradiography.

Exonuclease Assays. Enzyme solutions were diluted in 20 mM MOPS–NaOH (pH 7.5), 0.1 mM EDTA, 10% glycerol (v/v), 100 mM NaCl, 1 mM DTT, acetylated BSA (0.1 mg/mL). Unless stated differently, amounts of substrate DNA are given in nanomoles of nucleotides. Immediately prior to the assay, the substrate DNA was boiled for 5 min and chilled in ice water. Standard reaction mixtures contained 1 nmol of *Hae*III-cleaved [^{3}H]T7 DNA, 25 mM ethanolamine–acetic acid (pH 9.3), 10 mM $Mg(OAc)_2$, 0.5 mM DTT, and acety-

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[1] Abbreviations: RFI DNA, replicative form DNA; ds DNA, double-stranded DNA; ss DNA, single-stranded DNA; DTT, dithiothreitol; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PMSF, phenylmethanesulfonyl fluoride; TCA, trichloroacetic acid: TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; BSA, bovine serum albumin.

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Table I: Purification of *S. pombe* ExoII[a]

step	total protein (mg)	volume (mL)	total act. (units)	sp act. (units/mg)	purification (x-fold) (total/step)	yield (%) (total/step)
I (crude extract)	1800	170	590	0.33	1	100
II (polyethylenimine/$(NH_4)_2SO_4$)	1100	27	490	0.45	1.4	82
III (DNA–cellulose)	19	13	260	14	42/31	45/54
IV (Sephacryl S-200)	6.1	3.4	140	22	67/1.6	23/51
V (Mono Q)	0.26	1.9	60	230	700/11	10/39

[a] From 70 g of meiotically induced cells (approximately 3×10^{11} cells) as described in Materials and Methods.

lated BSA (0.1 mg/mL) in 50 μL and were incubated at 31 °C for 10 min. The reactions were stopped by adding 25 μL of calf thymus DNA (0.2 mg/mL) and 250 μL of 5% TCA and kept on ice for 10 min. After centrifugation for 5 min in a microcentrifuge, 260 μL of the supernatant fluid was carefully removed and mixed with 3 mL of scintillation fluid (Ready Safe; Beckman Instruments, Palo Alto, CA). Radioactivity was measured in a Beckman LS3801 scintillation counter. Reactions with a volume of 0.5 mL were precipitated with 50 μL of 50% TCA, and 4 mL of scintillation fluid was used. One enzyme unit was defined as the activity rendering 1 nmol of nucleotides TCA-soluble in 1 min. Exonuclease reactions were carried out in duplicate, and the counts measured were within 10% of each other.

Purification of Exonuclease II. *S. pombe* strain GP535 (*h⁻ ade6-M26 pat1-114 end1-458*) was grown and thermally induced to undergo meiosis as described (Szankasi & Smith, 1992). Seventy grams of cells was disrupted, and fractions I and II were prepared as described (Szankasi & Smith, 1992; Table I). The following steps were carried out at 0–5 °C, and the buffers used were as described (Szankasi & Smith, 1992). Fraction II (1.1 g of protein) was applied to a ss DNA–cellulose column (U.S. Biochemicals, 1.6 $cm^2 \times 16.5$ cm) at 30 mL/h, and protein was eluted with a 110-mL linear gradient of NaCl from 120 mM to 500 mM into 4-mL fractions as described (Szankasi & Smith, 1992). Fractions containing ss DNA exonuclease activity, peaking at 250 mM, were pooled to give 12.5 mL of fraction III.

To fraction III was added 7 g of solid ammonium sulfate over a period of 10 min, while being stirred, and stirring was continued for 15 min. The precipitate was collected by centrifugation at 12000*g* for 20 min and redissolved in 0.7 mL of buffer B75 [20 mM MOPS–NaOH (pH7.5), 0.1 mM EDTA, 10% (v/v) glycerol, 1 mM DTT, 0.1 mM PMSF, TLCK (40 μg/mL), and aprotinin, leupeptin, and pepstatin (1μg/mL)]. The solution was chromatographed on a Sephacryl S-200 column (Pharmacia LKB Biotechnology Inc., 1 × 48 cm) at 3 mL/h in buffer B75 containing 120 mM NaCl, and 0.85-mL fractions were collected. Two microliters of 20-fold dilutions were assayed for enzyme activity. Fractions containing ss DNA exonuclease activity, which eluted at 39% of the total column volume, were pooled to give 3.4 mL of fraction IV.

Fraction IV was diluted with an equal volume of buffer B75 and applied onto a Mono Q column (HR5/5, Pharmacia LKB Biotechnology Inc.) at 0.5 mL/min, and 1-mL fractions were collected. The column was rinsed with 20 mL of buffer B75 containing 60 mM NaCl. After a 5-mL gradient of 60–170 mM NaCl in buffer B75 was applied, the column was washed with 20 mL of buffer B75 containing 170 mM NaCl. ss DNA exonuclease activity was eluted with a 15-mL gradient of 170–270 mM NaCl in buffer B75. Two microliters of 20-fold dilutions was assayed, and peak fractions were pooled to give 1.9 mL of fraction V.

Fraction V was dialyzed against buffer C [20 mM MOPS–NaOH (pH 7.5), 0.1 mM EDTA, 50% (v/v) glycerol, 100 mM NaCl, 1 mM DTT] for 10 h, and 10-μL aliquots were frozen in liquid N_2 and stored at −70 °C.

Samples of ExoII frozen and stored as described above suffered an initial 3-fold loss in specific activity but were then stable for at least one year.

Protein Analysis. Protein concentrations were determined according to Bradford (1976), using the protein assay dye purchased from Bio-Rad and BSA as a standard. Protein samples were separated by $NaDodSO_4$–polyacrylamide gel electrophoresis (Laemmli, 1970) on a 0.75-mm 7.5% gel in a Mighty Small apparatus (Hoefer) and stained with Coomassie Brilliant Blue R-250. Samples in large volumes were concentrated by precipitation in 10% TCA prior to gel electrophoresis.

RESULTS

Identification and Purification of a ss DNA Exonuclease. For the detection of exonucleases in *S. pombe*, we used a strain (GP535) which lacks, due to the *end1-458* mutation, a potent endonuclease (Uemura & Yanagida, 1984). This strain is devoid of any detectable ds or ss DNA endonuclease activity (data not shown); the remaining nuclease activity, measured as acid-solubilization of linear uniformly ^3H-labeled DNA, is therefore exonucleolytic. The T7 DNA substrate was cleaved with the restriction endonuclease *Hae*III into 69 fragments in order to ensure an excess of DNA ends over enzyme molecules in the reactions. Strain GP535 also carries the conditional *pat1-114* mutation and undergoes meiosis at the restrictive temperature, bypassing the requirements of nitrogen starvation and mating-type heterozygosity (Iino & Yamamoto, 1985; Beach et al., 1985). Cells were induced to undergo synchronous meiosis and harvested after 4.75 h as described earlier (Szankasi & Smith, 1992) and in Materials and Methods. Under these conditions, the bulk of premeiotic DNA replication occurs between 1.5 and 3 h, and the meiotic divisions occur between 5 and 6.5 h after shift to the restrictive temperature (Szankasi & Smith, 1992). Initial characterization of exonuclease activities in crude extracts from meiotic cells revealed activity on both ss and ds DNA substrates (data not shown).

Table I summarizes the purification of the ss DNA exonuclease activity, which we have named exonuclease II (ExoII). ExoII was largely separated on the DNA cellulose column from ExoI, which elutes at 320 mM NaCl (see Materials and Methods; Szankasi & Smith, 1992). ExoII was purified 700-fold and has a M_r of 134 000 as estimated by $NaDodSO_4$–polyacrylamide gel electrophoresis (Table I; Figure 1A). Aliquots of the Mono Q column fractions at and around the peak of enzyme activity were run on a gel, and the intensity of the M_r 134 000 band paralleled the amount of enzyme activity measured in each fraction (Figure 1B). By estimating the M_r 134 000 band in Figure 1A, lane V, to contain 200 ng of protein, we calculated that there are approximately 10 000 ExoII molecules per meiotic cell.

Exonuclease II Specifically Degrades ss DNA in the 5′→3′ Direction. To evaluate the specificity of ExoII for ss DNA,

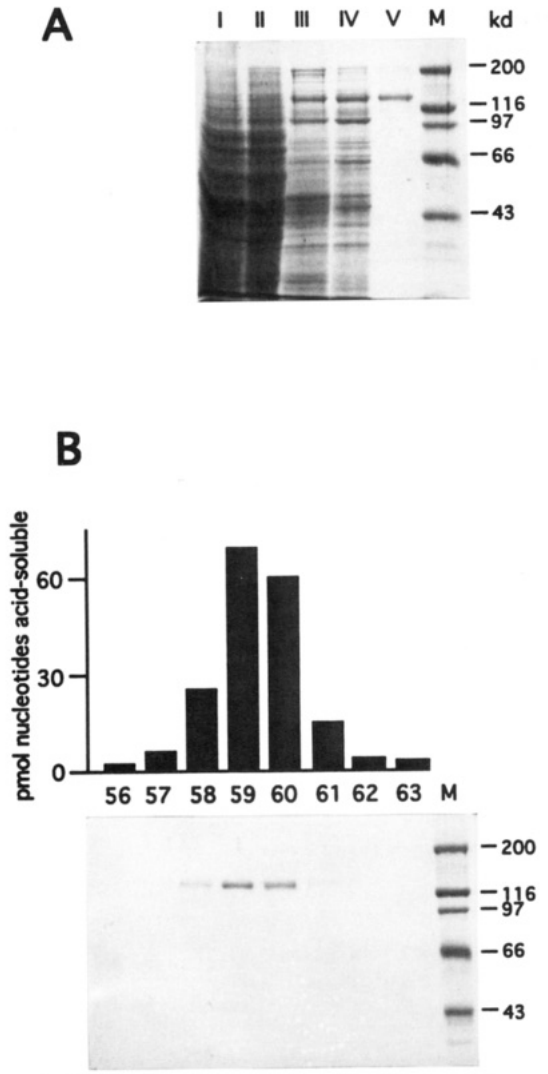

FIGURE 1: Electrophoretic separation of purified protein samples. (A) Aliquots from fractions I through V (see Materials and Methods, Table I) were run on a 7% $NaDodSO_4$–polyacrylamide gel as indicated. The following amounts were loaded: I, 52 μg (0.02 unit of ExoII); II, 60 μg (0.02 unit); III, 11 μg (0.15 unit); IV, 7 μg (0.16 unit); V, 0.7 μg (0.19 unit); M, size standards (high range, Bio-Rad), 0.5 μg each, with their molecular weights indicated. (B) (top) 0.1 μL of fractions 56 to 63 from the Mono Q column was assayed for ss DNA exonuclease activity as described in Materials and Methods, and the results are displayed in this bar graph. (bottom) Four microliters of the same fractions was run on a 7% $NaDodSO_4$–polyacrylamide gel. Lane M contains size standards. Numbers in the middle indicate fraction numbers. Fractions 59 and 60 were pooled to give fraction V.

purified enzyme was reacted with native or boiled *Hae*III-cleaved [^{3}H]T7 DNA. Figure 2 shows that ss DNA was rendered acid-soluble approximately 50–100-fold more rapidly than ds DNA. The reaction on heat-denatured (ss) DNA was linear over a period of 20 min, followed by a gradual loss of activity. This loss is not due to renaturation of the substrate, since preincubation of heat-denatured DNA for various times did not affect digestion by subsequently added enzyme (data not shown). ExoII might be slightly unstable under the assay conditions or inhibited by the digestion products.

Assuming 9 pmol of ExoII molecules per enzyme unit (see estimate above and Table I) and all enzyme molecules to be active, we calculated that the rate of digestion was about 120 phosphodiester bonds/min per enzyme molecule for the first 20 min.

We next addressed the question of whether ExoII has a directionality of digestion. Uniformly ^{3}H-labeled *Hae*III-cleaved T7 DNA was mixed with DNA ^{32}P-labeled at either the 5′ or the 3′ end and reacted with enzyme. The 3′ ends appeared to be resistant to digestion by ExoII (Figure 3A, open circles) but the 5′ ends were readily rendered acid-soluble (Figure 3B, open squares). The nearly equal amounts of ^{3}H-labeled material released (Figure 3, closed symbols) confirmed comparable activity of ExoII in the two experiments. We conclude that ExoII digests ss DNA in the 5′→3′ direction.

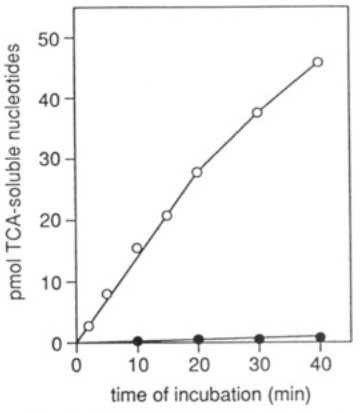

FIGURE 2: Single-strand specific action by ExoII. ExoII (1.4×10^{-3} units) was reacted with 0.5 nmol of native (●) or heat-denatured (○) *Hae*III-cleaved [^{3}H]T7 DNA for the times indicated, and acid-soluble nucleotides were measured, as described in Materials and Methods.

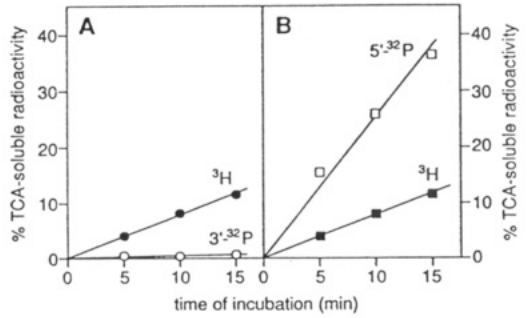

FIGURE 3: Polarity of digestion by ExoII. A total of 1 nmol of *Hae*III-cleaved [^{3}H]T7 DNA was reacted with ExoII (8.0×10^{-3} unit) for the times indicated and analyzed as described in Materials and Methods. The reactions contained, in addition, 5000 cpm of linear DNA labeled with ^{32}P (see Materials and Methods) at the 3′ end (A) or the 5′ end (B).

Table II: Cofactor requirements of ExoII

reaction condition	activity (% of standard)	reaction condition	activity (% of standard)
standard reaction[a]	100	+$Ca(OAc)_2$ at 20 mM	0.5
−$Mg(OAc)_2$	0.5	+$Zn(OAc)_2$ at 0.05 mM	83
−$Mg(OAc)_2$, + $Mn(OAc)_2$ at 10 mM	16	+$Zn(OAc)_2$ at 0.1 mM	78
−$Mg(OAc)_2$, + $MgCl_2$ at 10 mM	118	+$Zn(OAc)_2$ at 0.5 mM	2
−DTT[b]	58	+NaCl at 50 mM	94
−BSA	61	+NaCl at 100 mM	65
+ATP at 1 mM	94	+NaCl at 200 mM	11
+$Ca(OAc)_2$ at 5 mM	23	+0.5 μg of RNA	104
+$Ca(OAc)_2$ at 10 mM	10	+1.0 μg of RNA	102

[a] As described in Materials and Methods using 3.5×10^{-3} units of ExoII. [b] Enzyme diluted in buffer lacking DTT.

The cofactor requirements of ExoII are shown in Table II. ExoII required a divalent cation, with Mg_2^+ being favored over Mn^{2+}. Ca^{2+} and Zn^{2+} were inhibitory, with full inhibition at 20 mM and 1 mM, respectively. As counterions OAc^- or Cl^- gave similar results. ExoII activity was also salt-sensitive, being reduced 10-fold upon the addition of 200 mM NaCl. The addition of ATP to 1 mM had no effect, whereas DTT

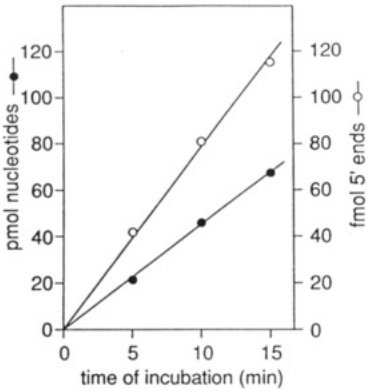

FIGURE 4: Processivity of ExoII. Ten micrograms of full-length [^{3}H]T7 DNA (730 fmol of 5′ ends) and approximately 50 ng of full-length [^{3}H]T7 DNA labeled with ^{32}P at the 5′ ends were reacted with ExoII (4.2×10^{-3} unit or 58 fmol) for the times indicated and analyzed as described in Materials and Methods. The percentage of ^{32}P label rendered acid-soluble was assumed to reflect the percentage of 5′ ends rendered acid-soluble.

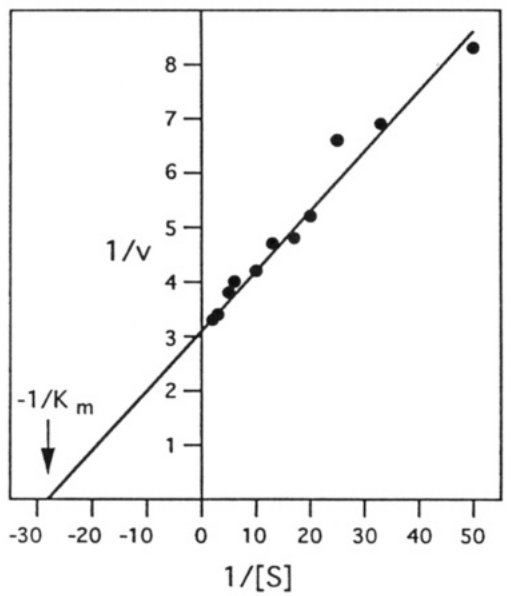

FIGURE 5: Substrate affinity for ExoII. ExoII (6.3×10^{-4} unit, final concentration 10 pM) was reacted with various amounts of full-length [^{3}H]T7 DNA in 0.5-mL reactions for 20 min and analyzed as described in Materials and Methods. The results are displayed in a double-reciprocal Lineweaver–Burk plot; [S] is the nanomolar concentration of 5′ ends and v is the velocity in 39 pmol of nucleotides rendered TCA-soluble in 20 min. The K_m is estimated to be 36 pM.

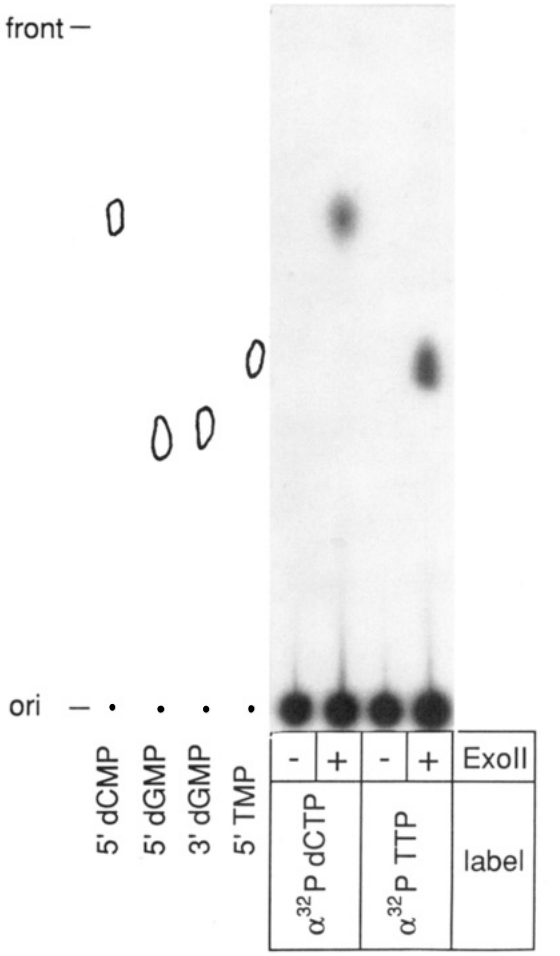

FIGURE 6: Analysis of reaction products by thin-layer chromatography. Unlabeled nucleotide standards (left four lanes) and ExoII digestion products from internally ^{32}P-labeled DNA (right four lanes) were chromatographed from bottom to top as described in Szankasi and Smith (1992). The tracing shows the positions of the unlabeled nucleotide standards seen as dark spots on the chromatography plate under UV light. The DNA substrates internally labeled with either [α-^{32}P]dCTP or [α-^{32}P]TTP were analyzed after incubation with or without ExoII as indicated. Substrate derived from synthesis with 1 μg of M13mp18 template DNA (see Materials and Methods) was reacted with ExoII (3.5×10^{-3} unit) under standard reaction conditions.

and BSA appeared to be slightly stimulatory. Unlabeled RNA present in a 10–100-fold excess with respect to ends (estimated from its average length; data not shown) did not compete significantly with the DNA substrate. We therefore conclude that ExoII is DNA-specific. Maximal activity was observed at pH 9.3, with ethanolamine–acetic acid being favored over a sodium carbonate buffer (data not shown). At pH 7.5 and 10.0, activity was reduced to 25% and 10%, respectively (data not shown).

Exonuclease II Acts Processively. To further elucidate the mechanism of digestion by ExoII, we estimated the average extent of digestion on one DNA molecule before the enzyme dissociates. We attempted this by reacting ExoII with uniformly ^{3}H-labeled and 5′ ^{32}P-end-labeled T7 DNA with an excess of DNA ends over enzyme molecules, a method devised by A. Johnson and R. Kolodner (personal communication). The rationale of this experiment is that, during a time course, dissociation of the enzyme from one substrate molecule and reinitiation on another substrate molecule will result in the release of ^{32}P label proportional to the number of ends attacked (as long as there is an excess of undigested ends), whereas the overall amount of digestion is measured by the release of ^{3}H label. The ratio of the moles of total ^{3}H-labeled nucleotides rendered acid-soluble to the moles of 5′ ^{32}P-end-labeled nucleotide ends rendered acid-soluble should represent the average number of nucleotides digested before dissociation of the enzyme. In Figure 4, we show the release of acid-soluble internal ^{3}H-labeled nucleotides (closed circles) and 5′ ^{32}P end label (open circles) for the times indicated. The average ratio between the two values was 560. Therefore, ExoII processively digests approximately 560 nucleotides on average from one DNA molecule before it dissociates. To confirm the presence of a sufficiently large excess of undigested ends, the 10-min incubation was repeated with twice as much and half as much enzyme. In both cases similar values, 580 and 510, respectively, were obtained.

To obtain an estimate of the affinity of ExoII for its substrate, we determined its K_m for DNA ends. Ten picomolar ExoII was reacted with varying amounts of [^{3}H]T7 DNA with concentrations of 5′ ends ranging between 20 and

500 pM. Due to the low amounts of enzyme, the reactions were scaled up to 0.5 mL and incubated for 20 min. Figure 5 shows the results displayed in a double-reciprocal Lineweaver–Burk plot, from which we estimated an apparent K_m of 3.6×10^{-11} M 5′ ends.

Exonuclease II Produces 5′ Mononucleotides. The products of ExoII digestion were determined by digesting internally ^{32}P-labeled substrate DNA obtained by synthesizing the strand complementary to ss M13mp18 DNA with either [α-^{32}P]-dCTP or [α-^{32}P]TTP as the labeled nucleoside triphosphate. The rationale of this experiment was as follows. If the enzyme released 5′ mononucleotides, then the ^{32}P atoms would be released with the same nucleotide used for synthesis. If, on the other hand, 3′ phosphates were produced, then the ^{32}P atoms would be released with the nucleotide to the 5′ side of the one originally used for labeling, and all dNMP's in the reaction products would be labeled. Figure 6 shows the analysis of reaction products by thin-layer chromatography. In the digestion products, ^{32}P label was present only in dCMP when dCTP was used for labeling and only in TMP when TTP was used for labeling. In addition, product formation was quantitated in two ways. An aliquot of the reactions was precipitated with TCA (as for standard exonuclease reactions), and the radioactivity in the soluble and insoluble fraction was determined. From the chromatogram, the spots containing digestion products and the origin were cut out, and radioactivity was determined. The measurements gave 6–8% acid-soluble radioactive material and free nucleotides, respectively. This argues against ExoII producing short acid-soluble oligonucleotides that remain at the origin of the chromatogram. We conclude that ExoII produces 5′ mononucleotides.

DISCUSSION

We have purified a DNA exonuclease from meiotic cells of *S. pombe*. The enzyme, ExoII, specifically degraded ss DNA in the 5′→3′ direction producing 5′ mononucleotides at a rate of 120 nucleotides/min. It had a high affinity for ss DNA ends, with an apparent K_m of 3.6×10^{-11} M.

We are interested in the role of eukaryotic exonucleases in DNA damage repair and meiotic recombination. To obtain synchronous meiotic cultures of *S. pombe*, we have utilized the conditional *pat1-114* mutant, which undergoes rapid meiosis at the restrictive temperature bypassing the requirements of nitrogen starvation and mating-type heterozygosity for meiosis of *pat*$^+$ cells (Iino & Yamamoto, 1985; Beach et al., 1985; Szankasi & Smith, 1992). A 5′ exonuclease specific for ds DNA, ExoI, is induced at 4.75 h after heat induction of meiosis in a *pat1-114* mutant (Szankasi & Smith, 1992). This time is in the period when recombination occurs, between premeiotic S-phase and the meiotic divisions. In this paper, we have described the purification and characterization of a ss DNA exonuclease, ExoII, present in extracts from meiotic cells. Total ss DNA exonuclease activity does not significantly change during the induction of meiosis (data not shown), but we do not know whether the meiotic and mitotic activities are identical.

ExoII specifically degraded ss DNA in the 5′→3′ direction. This mode of degradation is similar to that of the *E. coli* RecJ protein (Lovett & Kolodner, 1989). However, one RecJ molecule produces approximately 1000 nucleotides/min, as compared to 120 by ExoII. RecJ is required for both UV resistance and recombination by the RecF pathway (Lovett et al., 1988; Lloyd et al., 1988; Lovett & Clark, 1984). By analogy, it is likely that *S. pombe* ExoII is involved in similar functions. One possibility is that, after the action of a helicase from a double-strand break, ExoII degrades the 5′ ending strand. The exposed 3′ single strand could be a substrate for strand invasion with a homologous DNA duplex. Another possibility is that ExoII creates a single-strand gap after the action of a helicase initiating at a nick. Such a function could be involved in the repair of mismatched base pairs. ExoII could also act at a later step such as the removal of displacement loops after strand invasion or the trimming of excess single-strand tails to create ligatable nicks.

With an apparent K_m of 3.5×10^{-11} M, ExoII has a very high affinity for its substrate. Since the *S. pombe* nuclear diameter is roughly 2 μm (Robinow & Hyams, 1989), one ss DNA 5′ end per nucleus is equivalent to 4×10^{-10} M, or 10 times the K_m, and would be readily bound by ExoII. We have estimated that there are approximately 10 000 ExoII molecules per cell. This large amount and the very low K_m for ss DNA 5′ ends may reflect another function of the protein. We have cloned the genes for both ExoI and ExoII using partial amino acid sequences of the proteins (unpublished results). These clones may allow construction of *S. pombe* mutants lacking these enzymes and further investigation of their function in *S. pombe*.

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