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# Partial purification of an enzyme from *Saccharomyces cerevisiae* that cleaves Holliday junctions*

**(cruciform structures/figure-8 molecules/genetic recombination)**

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ABSTRACT **An enzyme from *Saccharomyces cerevisiae* that cleaves Holliday junctions was partially purified ≈500- to 1000-fold by DEAE-cellulose chromatography, gel filtration on Sephacryl S300, and chromatography on single-stranded DNA-cellulose. The partially purified enzyme did not have any detectable nuclease activity when tested with single-stranded or double-stranded bacteriophage T7 substrate DNA and did not have detectable endonuclease activity when tested with bacteriophage M13 viral DNA or plasmid pBR322 covalently closed circular DNA. Analysis of the products of the cruciform cleavage reaction by electrophoresis on polyacrylamide gels under denaturing conditions revealed that the cruciform structure was cleaved at either of two sites present in the stem of the cruciform and was not cleaved at the end of the stem. The cruciform cleavage enzyme was able to cleave the Holliday junction present in bacteriophage G4 figure-8 molecules. Eighty percent of these Holliday junctions were cleaved in the proper orientation to generate intact chromosomes during genetic recombination.**

It has been proposed that genetic recombination often involves the formation of an intermediate structure that contains a reciprocal single-stranded crossover between two homologous duplexes, the Holliday junction (1–3). When the participating genomes are circular, the Holliday intermediate has a figure-8 configuration. DNA molecules containing Holliday structures have been observed in recombining phage and plasmid DNA molecules isolated from *Escherichia coli* and yeast cells and in chromosomal DNA isolated from yeast (4–10). The enzymology of the formation and resolution of Holliday structures is not well understood, but the RecA and RecI proteins, from *E. coli* and *Ustilago maydis*, respectively, probably play a role in the formation of Holliday junctions in these organisms (11, 12). Resolution of the Holliday junction requires cleavage of the crossed strands, realignment, and ligation to generate an intact recombinant duplex. The bacteriophage T7 gene *3* and bacteriophage T4 gene *49* endonucleases cleave artificially constructed Holliday structures (13, 14). Conditionally lethal mutations in these genes lead to an accumulation of highly branched DNA after infection, suggesting that their gene products may resolve branched recombination intermediates (13, 15–17). Artificial Holliday junctions containing the bacteriophage λ *att* sites within the crossover region are cleaved by the λ Int protein (18). The T4 and T7 enzymes differ from the Int protein in that they have endonuclease activity on single-stranded substrates and lack sequence specificity whereas the Int protein has no single-stranded DNA-specific endonuclease activity and only cleaves Holliday junctions constructed from λ *att* sites (14, 18, 19). We have recently demonstrated that DNA molecules having a figure-8 configuration are generated in a *Saccharomyces cerevisiae* cell-free recombination system and that these molecules appear to be processed during the reaction (20, 21). Here we describe an enzymatic activity from yeast that cleaves Holliday structures.

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## EXPERIMENTAL PROCEDURES

**Strains.** The *E. coli* strain JC10287 [Δ(*srlR–recA*)304, *thr-1, leu-6, thi-1, lacY1, galK2, ara-14, xyl-5, mtl-1, proA2, his-4, argE3, kdgK51, rpsL31, tsx-33, supE44*] used to propagate plasmids was obtained from A. J. Clark (University of California, Berkeley). An initial sample of pBR322::PAL114 DNA (22) was obtained from G. Warren (Advanced Genetic Sciences, Oakland, CA) and was used to transform *E. coli* JC10287 to yield *E. coli* RDK1567. A partial restriction map of this plasmid is presented in Fig. 1. The diploid yeast strain AP-1 (*MATa/MATα, ade1/ADE1, ade2-1/ade2-R8, ura1/URA1, his7/HIS7, lys2/LYS2, tyr1/TYR1, gal1/GAL1, CYH2/cyh2, CAN1/can1, LEU1/leu1*) was obtained from B. Byers (University of Washington, Seattle, WA) (23).

**Nucleic Acids.** Plasmid DNA was purified essentially as described (24). To convert pBR322::PAL114 DNA to the cruciform-containing form, it was incubated at 55°C for 1 hr in 10 mM Tris·HCl (pH 8.0)/1 mM EDTA/200 mM NaCl. This treatment converted 60–80% of the molecules to the *Bgl* II-resistant cruciform-containing form (22). To remove the cruciform structure the DNA was heated to 85°C for 5 min in 10 mM Tris·HCl (pH 8.0)/1 mM EDTA and quenched on ice. This converted >90% of the molecules to the *Bgl* II-sensitive non-cruciform-containing form (22). Bacteriophage T7 [$^3$H]DNA (44.6 cpm/pmol) was prepared as described (25). T7 DNA was denatured by incubating it at 100°C for 10 min followed by chilling on ice. Bacteriophage G4 figure-8 DNA (26) was a gift from R. C. Warner (Univ. of California, Irvine, CA). Salmon sperm DNA (type III) was from Sigma. Bacteriophage M13mp11 viral DNA was the gift of M. Howard of this laboratory. DNA concentrations are expressed in moles of nucleotide equivalents unless otherwise specified.

**Chemicals.** [*methyl*-$^3$H]Thymidine (80 Ci/mmol, 1 Ci = 37 GBq) was from New England Nuclear. [γ-$^{32}$P]ATP (3000 Ci/mmol) was from Amersham. Spermidine·HCl, dithiothreitol, and phenylmethylsulfonyl fluoride were from Sigma. Ultrapure Tris, ammonium sulfate, and ammonium acetate were from Schwarz/Mann. Zymolyase-100T and crystallized bovine serum albumin were from Miles. Media components

Abbreviation: kb, kilobase(s).

*A preliminary account of this work was presented at the 1984 Cold Spring Harbor Symposium on Genetic Recombination and at the 1984 International Conference on Yeast Genetics and Molecular Biology at Edinburgh (20).

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were from Difco. DEAE-cellulose (DE 52) was from Whatman and Sephacryl S300 was from Pharmacia. Single-stranded-DNA cellulose (0.98 mg/packed ml) was prepared as described (27).

**Assays.** The cruciform cleavage assay was carried out in 20 μl of 50 mM Tris·HCl (pH 7.8)/10 mM $MgCl_2$/1 mM dithiothreitol/50 μg of bovine serum albumin per ml/1.2 nmol of cruciform-containing pBR322::PAL114 DNA. After incubation at 30°C for 60 min, EDTA was added to 10 mM, and the DNA was purified by extraction with phenol and precipitation with ethanol. This DNA was digested with *Pvu* II or *Eco*RI and analyzed by agarose gel electrophoresis. This assay is illustrated in Fig. 1. In some experiments the reactions were stopped by heating to 65°C for 10 min followed by addition of either *Eco*RI or *Pvu* II and incubation at 37°C for 1 hr. The DNA was then analyzed by agarose gel electrophoresis. One unit of cruciform cleavage activity is defined as the amount of enzyme that cleaves 1 pmol of DNA molecules in 60 min at 30°C. Endonuclease assays were carried out using the same conditions except that 3 nmol of either M13mp11 viral DNA or pBR322 covalently closed circular DNA replaced the pBR322::PAL114 DNA and the restriction endonuclease digestion was omitted. The definition of endonuclease units is the same as that for the cruciform cleavage enzyme. Nuclease assays used the same conditions except 3 nmol of T7 DNA was present as substrate in a reaction volume of 50 μl. The reaction was stopped by the addition of 0.3 ml of salmon sperm DNA at 0.22 mg/ml and 0.3 ml of 1 M trichloroacetic acid at 0°C followed by centrifugation for 5 min in an Eppendorf microcentrifuge at 4°C. The acid-soluble radioactive material was quantitated by adding 0.4 ml of the supernatant to 4 ml of aqueous scintillation fluid and counting. One unit of nuclease activity will produce 1 nmol of acid-soluble nucleotides in 60 min at 30°C. Protein concentrations were determined by using the Lowry assay and bovine serum albumin as a standard (28).

**Enzymes.** Restriction endonucleases were obtained from New England Biolabs and were used according to instructions provided. T4 polynucleotide kinase and bacterial alkaline phosphatase were purified as described (29, 30).

**Analysis of Plasmid DNA.** Electrophoresis was carried out in 0.8% agarose slab gels with 40 mM Tris/5 mM acetate/1 mM EDTA/ethidium bromide (0.5 μg/ml), pH 7.9, or under denaturing conditions in 12% polyacrylamide gels with 90 mM Tris/90 mM borate/2.5 mM EDTA/6 M urea (pH 8.3) (24, 31). DNA samples were incubated at 100°C for 2 min prior to electrophoresis on acrylamide gels. Dephosphorylation with bacterial alkaline phosphatase and 5′-end labeling with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase were carried out as described (30). Electron microscopy was carried out as described (24). DNA was purified from excised gel slices by using the "freeze and squeeze" method (32).

**Purification of the Cruciform Cleaving Enzyme.** *S. cerevisiae* strain AP-1 was grown to $5 \times 10^7$ cells per ml in yeast extract/peptone/dextrose broth, harvested by centrifugation, resuspended in 50 mM Tris·HCl (pH 7.5)/10% (wt/vol) sucrose/1 mM EDTA at $2.5 \times 10^9$ cells per ml and stored at −70°C as described (21, 33). Cells (160 ml) were thawed at room temperature and placed on ice, and the following additions were made: 4 M KCl to a final concentration of 400 mM, 0.1 M spermidine (pH 8.0) to a final concentration of 5 mM, 0.5 M EDTA (pH 8.0) to a final concentration of 1 mM, 2-mercaptoethanol to a final concentration of 14.3 mM, and 10 mg of Zymolyase 100T per ml to a final concentration of 0.4 mg/ml. After 90 min on ice, 0.1 M phenylmethylsulfonyl fluoride and 10% (vol/vol) Brij-58 were added to final concentrations of 0.1 mM and 0.1%, respectively. Incubation on ice continued for 20 min. This lysate was centrifuged at 30,000 rpm for 45 min in a Beckman Ti60 rotor at 4°C, and the supernatant was saved (fraction I, 190 ml). Ammonium sulfate (66.7 g) was added to fraction I (190 ml) while it was stirred on ice, over 30 min. After stirring for an additional 30 min, the solution was centrifuged at 15,000 rpm for 10 min in a Sorvall SS-34 rotor at 4°C, and the pellet was suspended in buffer A [20 mM Tris·HCl (pH 7.5)/0.1 mM EDTA/10 mM 2-mercaptoethanol/10% (wt/vol) glycerol/0.1 mM phenylmethylsulfonyl fluoride] (fraction II). The concentration of ammonium sulfate in fraction II was reduced to below 50 mM by dialysis against two 2-liter changes of buffer A over 3 hr at 0°C. This fraction was applied to a DEAE-cellulose column (12.6 $cm^2 \times 14$ cm) equilibrated with buffer A containing 50 mM NaCl. The column was washed with 175 ml of the same buffer, and the proteins were eluted with 1.5 liters of a linear gradient from 50 mM to 500 mM NaCl in buffer A. The enzymatic activity eluted between 150 and 195 mM NaCl. The active fractions were pooled and the proteins were precipitated with ammonium sulfate (352 g/liter) as described above and suspended with 2 ml of buffer A (fraction III). Fraction III was layered onto a column of Sephacryl S300 (1.77 $cm^2 \times 50$ cm) that had been equilibrated with buffer A containing 300 mM NaCl, and the column was eluted with the same buffer. The enzymatic activity eluted at approximately 0.6 column volume, and the active fractions were pooled (fraction IV). Fraction IV was diluted with 2 vol of buffer A and applied to a column of single-stranded DNA-cellulose (0.64 $cm^2 \times 8$ cm) equilibrated with buffer A containing 100 mM NaCl. The column was washed with 5 ml of the same buffer, and the proteins were eluted with 55 ml of a linear gradient from 0.1 to 0.7 M NaCl in buffer A. The enzymatic activity eluted between 400 and 500 mM NaCl. The active fractions were pooled and diluted with sufficient buffer A to lower the NaCl concentration to 100 mM. This solution was applied to a DEAE-cellulose column (0.64 $cm^2 \times 1.6$ cm) that had been equilibrated with buffer A containing 100 mM NaCl, and the flow-through fractions were collected. These fractions were concentrated to 0.6 ml by ultrafiltration using an Amicon PM10 membrane, diluted with 0.6 ml of glycerol, and stored at −20°C (fraction V).

## RESULTS

**Assay Systems.** We have used two model substrates containing Holliday junctions in assays designed to detect yeast enzymes that cleave Holliday junctions. The first assay utilizes the observation that plasmid DNAs containing a palindromic sequence treated to extrude a cruciform structure contain a Holliday junction at the base of the cruciform (13, 14, 22, 34, 35). We have used the plasmid pBR322::PAL114. This plasmid contains two copies of a 57-base-pair repeat inserted in inverted orientation into the unique *Bam*HI site of pBR322 such that it contains a single *Bgl* II site at the center of the palindrome (22). When the cruciform is extruded, this *Bgl* II site is resistant to digestion by *Bgl* II, and this can be used to detect the presence or absence of the cruciform. A map of pBR322::PAL114 containing the extruded cruciform is presented in Fig. 1. When this DNA is cleaved diagonally across the Holliday junction in either of the possible orientations monomer-length linear molecules containing hairpin ends at a specific site will result (Fig. 1). Cleavage of these linear molecules with a restriction endonuclease such as *Pvu* II that cleaves them at one unique site will yield two unique DNA fragments that can be detected by electrophoresis on agarose gels. This assay can be used to distinguish between endonucleases that make double-strand breaks at the site of the cruciform and other nonspecific endonucleases. The use of this assay to detect a yeast cruciform cleavage enzyme during chromatography on DEAE-cellulose is presented in Fig. 2.

The second assay that we have used utilizes figure-8 DNA molecules as substrates. These DNA molecules were originally detected as naturally occurring dimers that contained a Holliday junction, and subsequently methods for their

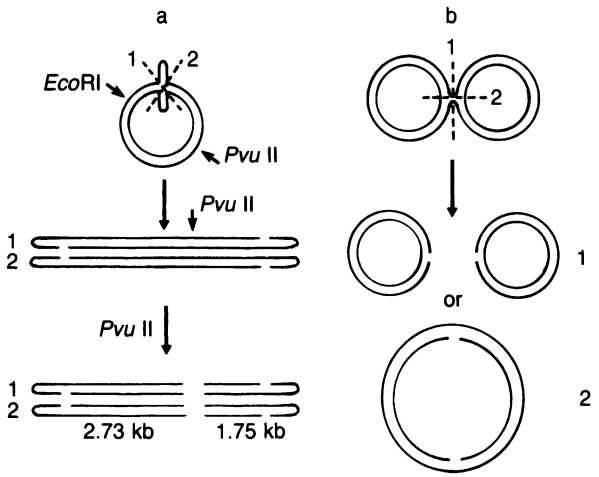


FIG. 1. Illustration of assays for Holliday junction cleavage. (*a*) Partial map of pBR322::PAL114 containing an extruded cruciform structure. Cleavage of the Holliday junction in either orientation, 1 or 2, yields the products labeled 1 and 2, respectively. Subsequent digestion with *Pvu* II will yield two fragments 2.73 kilobases (kb) and 1.75 kb long. Note that there is a *Hin*dIII site 23 base pairs in from the *Eco*RI site toward the cruciform, although this is not indicated in the figure. (*b*) Structure of a figure-8 molecule. Cleavage of the Holliday junction in orientation 1 or 2 will yield circular monomers or dimers, respectively.

construction have been developed (26, 36). The structure of a figure-8 molecule is illustrated in Fig. 1. Cleavage of this DNA diagonally across the Holliday junction will yield either circular monomers or circular dimers. The conversion of figure-8 molecules to circular monomers or dimers can then be detected either by electrophoresis on agarose gels or by electron microscopy.

**Purification of the Cruciform Cleaving Enzyme.** The cruciform cleavage enzyme used in these studies was purified from logarithmic-phase AP-1 yeast cells. The elution profile of the cruciform cleaving activity obtained during chromatography on DEAE-cellulose is presented in Fig. 2. We estimate that our purification procedure resulted in a 500- to 1000-fold purification of the cruciform cleavage enzyme with a yield of about 5%, although it was difficult to accurately estimate the amount of activity present in either the crude extract or the ammonium sulfate fraction. The final enzyme preparation had a specific

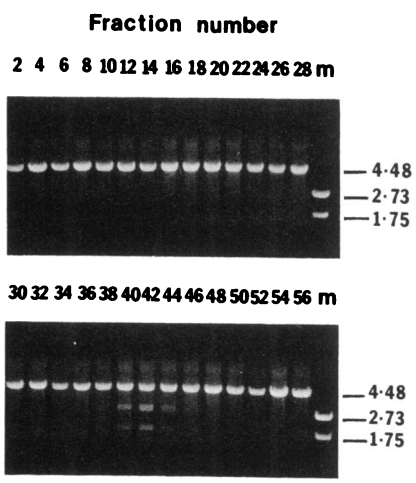


FIG. 2. Results obtained by assaying 1 μl of individual fractions obtained from the first DEAE-cellulose column for cruciform cleaving activity. The size markers (m, in kb) are pBR322::PAL114 DNA digested with *Pvu* II and *Bgl* II.

activity of 350 units/mg. This enzyme preparation was also assayed for the presence of a number of nuclease activities. It had <5 units/mg of endonuclease activity on M13 viral DNA, <4 units/mg of endonuclease activity on pBR322 covalently closed circular DNA, <0.02 unit/mg of nuclease activity on double-stranded T7 DNA, and <0.02 unit/mg of nuclease activity on single-stranded T7 DNA.

**Characterization of the Cruciform Cleaving Activity.** When pBR322::PAL114 containing an extruded cruciform was incubated with fraction IV it was converted to a mixture of monomer length linear, and nicked and supercoiled circular DNA molecules (Fig. 3, lane 1). Digestion of this DNA with *Pvu* II produced monomer-length, 2.73-kb and 1.75-kb linear molecules (Fig. 3, lane 2). Digestion of the DNA with *Eco*RI produced monomer-length, 4.04-kb and 0.43-kb linear molecules (not shown). These data indicate that the linear molecules produced by incubation of pBR322::PAL114 having the cruciform structure with fraction IV were cleaved at the site of the cruciform. Substrate DNA lacking the cruciform structure was not cleaved specifically under these conditions (Fig. 3, lanes 3 and 4). This indicates that a preformed cruciform structure is required for cleavage. Identical results were obtained with fraction V, except that no circular DNA containing a single-strand interruption was produced because the last purification step removed the last traces of a contaminating nonspecific nicking enzyme (data not shown).

To determine the site of cleavage directly, pBR322::PAL114 having the cruciform structure was digested to ≈50% of completion with fraction V and then digested to completion with *Eco*RI. This DNA was labeled at the 5′ end and analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 4). Only the DNA fragments derived from the short interval between the *Eco*RI site and the cruciform were analyzed, and only the relevant portion of the gel is presented because the gel system used was incapable of resolving the DNA fragments derived from the large interval between the *Eco*RI site and the cruciform. If cleavage at the single-stranded end of the extruded cruciform had occurred, then only a single 432-nucleotide DNA fragment would be observed. Instead, four DNA fragments 485, 455, 405, and 370 nucleotides long were observed. This could have resulted if two populations of cruciform-containing molecules existed; one with the cruciform extruded all 57 base pairs and the other with the cruciform extruded 25 base pairs. Cleavage of the first population of molecules as illustrated in Fig. 1*a* would yield the 485- and 370-nucleotide fragments, and cleavage of the second population of molecules would yield the 455- and 405-nucleotide fragments. Two different combinations of labeling and cleavage could then lead to the visualization of all four DNA fragments:

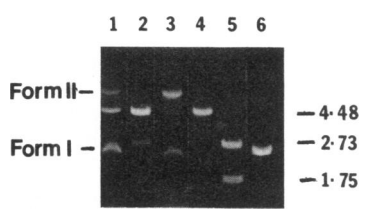


FIG. 3. Cleavage of cruciform DNA with fraction IV. When included, fraction IV was present at 0.2 unit per 20-μl assay mix. Lane 1, cruciform-containing pBR322::PAL114 DNA treated with fraction IV. Lane 2, cruciform-containing pBR322::PAL114 DNA treated with fraction IV and then *Pvu* II. Lane 3, non-cruciform-containing pBR322::PAL114 DNA treated with fraction IV. Lane 4, non-cruciform-containing pBR322::PAL114 DNA treated with fraction IV and then *Pvu* II. Lane 5, non-cruciform-containing pBR322::PAL114 digested with *Pvu* II and *Bgl* II. Lane 6, cruciform-containing pBR322::PAL114 DNA without any enzyme treatment. Form I, covalently closed circular DNA; form II, circular DNA containing a single-strand interruption. The positions of the molecular size markers are at the right.

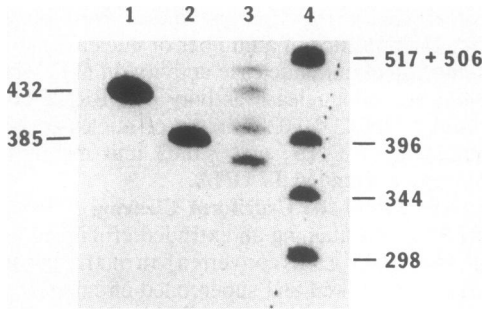


FIG. 4. Analysis of the products of cruciform cleavage by electrophoresis under denaturing conditions. Lane 1, 432-nucleotide marker made by digesting pBR322::PAL114 DNA with *Bgl* II and *Eco*RI. Lane 2, 385-nucleotide marker made by digesting pRDK35 DNA with *Eco*RI and *Bam*HI (37, 38). Lane 3, cruciform-containing pBR322::PAL114 DNA digested first with 0.3 unit of fraction V and then with *Eco*RI. Lane 4, marker fragments of the indicated sizes (nucleotides) made by digesting pRDK35 DNA with *Hin*fI (37, 38). All DNA fragments were labeled with $^{32}P$ at their 5′ ends and were visualized by autoradiography.

cleavage in both orientations combined with labeling at either the *Eco*RI site and/or the cruciform cleavage site, or cleavage in only one orientation combined with labeling at both the *Eco*RI site and the cruciform cleavage site. Digesting this DNA with *Hin*dIII to release the *Eco*RI end on a 23-nucleotide fragment indicated that labeling at all possible sites and cleavage in both orientations had occurred (data not shown).

**Resolution of Figure-8 Molecules.** A preparation of bacteriophage G4 figure-8 molecules (26) was digested with sufficient fraction IV to digest 50% of the pBR322::PAL114 DNA present in a standard assay and the reaction products were analyzed by agarose gel electrophoresis (Fig. 5*a*) and electron microscopy (Table 1 and Fig. 5*b*). Identical results were obtained with fraction V. Electron microscopy indicated that the starting figure-8 preparation was slightly contaminated with circular dimers, circular monomers, and linear monomers, and incubation with fraction IV converted the figure-8 molecules to circular monomers and dimers and a small proportion of $\alpha$ and $\sigma$ forms (Table 1 and Fig. 5*b*). The $\alpha$ forms consisted of a monomer-length circular molecule with two duplex tails, the sum of whose lengths equaled monomer length, attached to the same site, and the $\sigma$ forms consisted of a monomer-length circular molecule with an attached monomer-length duplex tail. Agarose gel electrophoresis showed that the figure-8 preparation contained one major species (Fig. 5*a*, lane 1, band B) and two minor species (Fig. 5*a*, lane 1, bands A and E). Incubation with the cruciform cleavage activity decreased the DNA present in band B concomitant with an increase in the DNA present in band A and the appearance of two new bands, C and D (Fig. 5*a*, lane 2). Electron microscopy indicated that band A contained circular dimers, band B contained figure-8 molecules, band C contained a mixture of $\sigma$ and $\alpha$ structures, band D contained circular monomers, and band E contained monomer-length linear molecules (Fig. 5*a*). These results are

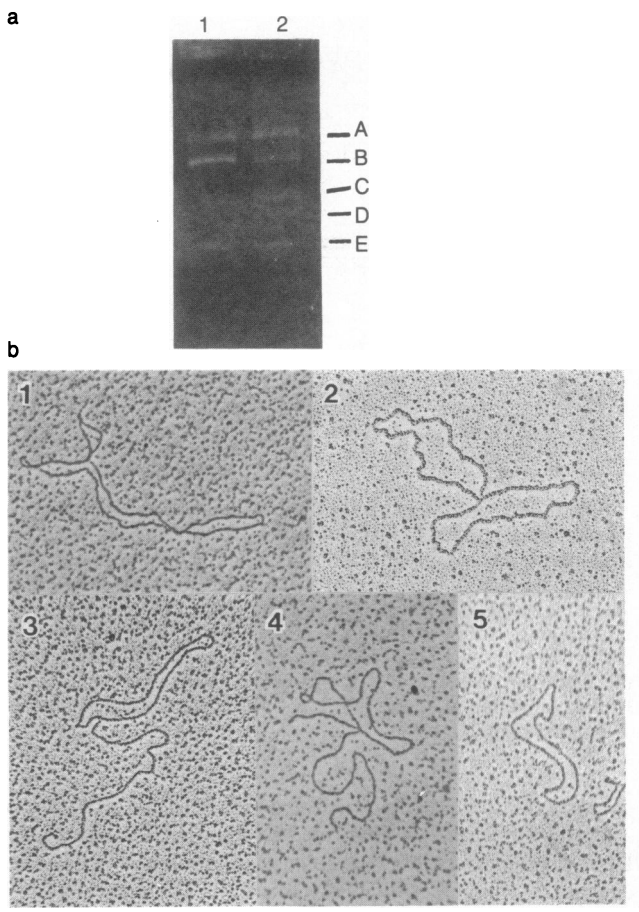


FIG. 5. Cleavage of bacteriophage G4 figure-8 molecules with fraction IV. Assay conditions were those described in the legend to Fig. 3 except that G4 figure-8 DNA was present as substrate DNA. (*a*) Electrophoretic analysis of figure-8 DNA before (lane 1) and after (lane 2) treatment with fraction IV. (*b*) Electron microscopic analysis of DNA molecules present in bands A–E. (*1*) Circular dimer from band A; (*2*) figure-8 from band B; (*3*) $\sigma$-form from band C; (*4*) $\alpha$-form from band C; (*5*) circular monomer from band D.

consistent with the cruciform cleavage activity cleaving the figure-8 molecules at the Holliday junction at a rate similar to the rate of cruciform cleavage. Furthermore, greater than 80% of the molecules that were cleaved were cleaved across the Holliday junction in the configuration required by recombination to generate intact chromosomes—in this case, circular monomers and dimers.

## DISCUSSION

In this communication we have described the preliminary characterization of an enzyme that was partially purified from yeast on the basis of its ability to specifically cleave plasmid DNA containing an extruded cruciform structure. Several lines of evidence suggest that this specific cleavage is due to the specific recognition and cleavage of Holliday junctions.

Table 1. Electron microscopic analysis of bacteriophage G4 figure-8 DNA digested with cruciform cleaving activity

| Incubation time, min | DNA species present, % | | | | | | |
|---|---|---|---|---|---|---|---|
| | Circular monomer | Circular dimer | Figure-8 | $\sigma$ form | $\alpha$ form | Linear monomer | *n* |
| 0 | 7.6 | 3.4 | 78.6 | 1.0 | — | 9.4 | 407 |
| 45 | 20.6 | 14.9 | 44.7 | 5.1 | 2.2 | 12.5 | 369 |

Enzyme reactions were exactly as described in the legend to Fig. 5. Representative electron micrographs are shown in Fig. 5*b*. *n*, Number of molecules present.

(*i*) The enzyme preparation did not contain any detectable endonuclease or exonuclease activity on single-stranded or double-stranded substrates that lack cruciform structures. The maximum levels of these activities that could be present in our enzyme preparation are insufficient to account for the cleavage of the cruciforms by activities such as S1 nuclease by at least a factor of 140 (39). (*ii*) The enzyme activity cleaved Holliday junctions in figure-8 molecules at the same rate that it cleaved cruciform structures even though the former structures are not thought to contain any unpaired nucleotides (40). That the figure-8 molecules were cleaved to yield mostly monomers and dimers indicates that cleavage most frequently occurred in the configuration required by recombination models to yield intact chromosomes. How $\alpha$ and $\sigma$ forms are produced is unclear; however, they could be due to aberrant 1,2 cleavage to yield $\sigma$ forms followed by branch migration to yield $\alpha$ forms. Future studies with homogeneous enzyme fractions and better defined substrates should provide insight into this reaction. (*iii*) Direct analysis of the products of the cruciform cleavage reaction indicated that the cruciform was cleaved specifically in the stem rather than in the single-stranded regions present at the ends of the stem as would have occurred with a single-strand-specific nuclease such as S1 nuclease (39). Our observation of two different specific cleavage sites could be explained if the relatively long cruciform used in these experiments could exist in two configurations that differ by the extent that the cruciform is extruded. How two populations of cruciforms might exist is unclear, but the presence of a region in the middle of the palindrome in which 9 of 11 base pairs are G·C base pairs (22) could act as a barrier against complete extrusion of the cruciform, and once the cruciform is fully extruded it could stabilize the fully extruded form. A second complication in interpreting this type of experiment is that since the base of the cruciform can move by branch migration and exist in different positions it is difficult to relate the positions of the cleavage sites to the position of the base of the cruciform at the time of cleavage.

In addition to the enzyme whose activity has been described here, three enzymes that can cleave Holliday junctions have been described. The λ Int protein is required for site-specific recombination and appears to only cleave and rejoin Holliday junctions constructed from *att* sites (18). The T7 gene *3* and T4 gene *49* proteins are required for genetic recombination, the maturation of DNA and, in the case of the T7 enzyme, the synthesis of precursors for DNA synthesis (13–19, 41). These latter two enzymes appear to cleave Holliday junctions without any sequence specificity. This activity may reflect the single-stranded DNA-specific endonuclease activity of these enzymes (14, 19). The yeast activity differs from the T4 and T7 enzymes in that it appears to lack detectable endonuclease and exonuclease activity other than its ability to cleave Holliday junctions. It differs from the λ Int protein in that it appears to cleave more than one type of Holliday junction, and it does not rejoin the broken phosphodiester bonds after the cleavage reaction (18). At present we have no information about the relationship between the yeast activity and any particular aspect of nucleic acid metabolism in yeast.

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