The Identification and Characterization of a G4-DNA Resolvase Activity*

(Received for publication, January 13, 1997, and in revised form, June 15, 1997)

Cindy Harrington‡, Yun Lan§, and Steven A. Akman¶

From the Department of Cancer Biology and Comprehensive Cancer Center of Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27157

There is increasing evidence that four-stranded Hoogsteen-bonded DNA structures, G4-DNA, play an important role in cellular processes such as meiosis and recombination. The Hoogsteen-bonded G4-DNA is thermodynamically more stable than duplex DNA, and many guanine-rich genomic DNA sequences with the ability to form G4-DNA have been identified. A protein-dependent activity that resolves G4-DNA into single-stranded DNA has been identified in human placental tissue. The resolvase activity was purified from any apparent nuclease activity and is dependent on NTP hydrolysis and MgCl₂. Resolvase activity is optimal with 5 mM MgCl₂. The Vₘₐₓ/Kₘ of ATP is 0.055%/min/μM, higher than the Vₘₐₓ/Kₘ of the other dNTPs. The products of the resolvase reaction are unmethylated single-stranded DNA. The resolvase is not a duplex DNA helicase or a topoisomerase II activity and does not unwind Hoogsteen-bonded triple helix DNA. Resolvase is a novel activity that unwinds stable G4-DNA structures using a dNTP-dependent mechanism producing unmethylated single-stranded DNA. Potential in vivo roles for this G4-DNA resolvase activity are discussed.

Guanine-rich DNA sequences that form G4-DNA are found in a number of evolutionarily conserved genomic regions such as telomeres, dimerization domains of retroviruses, and the insulin gene promoter (1–5). The four-stranded structure requires a monovalent cation to form, and the DNA strands can run in either a parallel or anti-parallel orientation (6–8). G4-DNA contains Hoogsteen bonds between the guanine residues forming square planar guanine quartets (9). X-ray crystal diffraction and two-dimensional nuclear magnetic resonance show the sugar backbone can exist in many variations (10–12). Guanine quartets have unusual stacking energy and high stability. The ability of the O-6 of guanine to form a coordination complex with either Na⁺ or K⁺ in guanine quartets is thought to stabilize telomeres (6–8). The thermodynamic parameters of parrelllel-stranded G4-DNA are indicative of its stability with the free energy of formation equal to −21 kcal/mol and the transition temperature above 82 °C (13). DNA sequences able to form G4-DNA have also been found at sites of spontaneous gene rearrangements, point mutations and, along with triplex DNA, have been implicated in causing DNA mutations (9, 14–16).

Many different proteins with specificity for binding to G4-DNA have been identified (17–22). The identification of a G4-DNA-specific nuclease from yeast as the SEP1/KEM1 protein, and the meiotic block at the 4N stage for KEM1-null cells, supports the hypothesis that G4-DNA is involved in meiosis (23, 24, 9). More recently, two yeast gene products with specific activity for G4-DNA were cloned and sequenced, G4p1 and G4p2 (19, 20). G4p1 is a homodimer of the gene encoding a novel protein with a domain homologous to the bacterial methionyl-tRNA synthetase dimerization domains, and G4p2 is encoded by a gene identical to genes that appear to function in protein kinase-controlled signal transduction, MPT4, and cell cycle progression, STO1 (19, 20). Recombinant MyoD, a transcription factor, also binds specifically to G4-DNA (21). The abundance of DNA sequences that have the ability to form G4-DNA and the possible link between G4-DNA, meiosis, transcription, and mutagenesis provide evidence not only for the formation of G4-DNA in vivo, but also for these high order DNA structures to play important roles in cellular metabolism.

In this paper we describe a novel G4-DNA metabolizing activity identified in human placental tissue. This activity is able to resolve four-stranded G4-DNA to single-stranded DNA. The activity was identified using G4-DNA formed from PZ33 as a substrate. PZ33 is an oligonucleotide (5'-AAAGTGATGGTG-GTGCGGGAAGGATCGAACCCT-3') with a nucleotide sequence that was identified as a hot spot for H₂O₂/Fe-mediated mutations in the supF gene of the reporter plasmid pZ189 (16, 36) and that has previously been shown to form G4-DNA in the presence of Na⁺ (16). Although the strand orientation of PZ33 in the G4-DNA form is not known with certainty, similar sequences have been documented to form parallel-stranded G4-DNA in the presence of Na⁺ (9). We have purified this activity from any contaminating nuclease, helicase, or topoisomerase II activities and have shown its optimal conditions along with its dependence on dNTP hydrolysis. This activity provides further evidence for the biological importance of G4-DNA, along with a potential mechanism for cells to handle bulky G4-DNA complexes that could disrupt DNA metabolic processes such as replication and transcription.

EXPERIMENTAL PROCEDURES

Materials—Radiolabeled ATP was from Amersham. T4 polynucleotide kinase was from Promega. The unlabeled dNTPs (ultrapure grade), DEAE-Sepharose CL-6B, Sephacryl S-200, and the Superdex-200 HR column were from Pharmacia Biotech Inc. ATPβS and bovine serum albumin were from Sigma. Hydroxyapatite resin and chelox-100 were from Bio-Rad. DNA oligonucleotides were synthesized in the Can-

---

* This work was supported by Council for Tobacco Research Grant 1571B and National Institutes of Health Grant CA-12197. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405.

§ Present address: Biochemical Sciences Dept., Amgen, Thousand Oaks, CA 91320.

¶ To whom correspondence should be addressed. Tel.: 910-716-0231; Fax: 910-716-0255; E-mail: sakman@bgsm.edu.

1 The abbreviations used are: ATP-βS, adenosine 5’-O-(thiotriphosphate); BME, β-mercaptoethanol; KP i, potassium phosphate buffer; kDNA, kinoplastic DNA; HIV, human immunodeficiency virus; ss, single-stranded.
DNA helicase II was a generous gift from Dr. Steve Matson (University of North Carolina). Human topoisomerase II and the topoisomerase II assay kit were from Topogen (Columbus, Ohio).

**DNA Substrates—G4-DNA was prepared by a modified method of Akman et al. (1991).** The G4 complex was formed by incubating 180 pmol/µl PZ33 in 20 mM Tris-HCl, pH 8.0, 0.2 mM EDTA, 250 mM NaCl at 10 °C for 10 min, followed by 10 min at 0 °C, and then 55 °C for 16 h. The DNA was electrophoresed on a 12% nondenaturing polyacrylamide gel at 4 °C and visualized by UV shadowing. The band containing greater than 90% of the total DNA in the G4 conformation. Marker DNAs were poly(dT) oligonucleotides of 36, 30, and 26 bases in length and a 5-mer (5'-AAAGT-3'). The M13/17-mer duplex helicase substrate was prepared by hybridizing 1 pmol of 5'-32P-labeled strat- agene 40 primer to ssM13mp9 in a 1:10 primer template ratio. The DNA was then passed through a Sephacyr S-200 spin column. The M13/17-mer tailed helicase substrate was prepared in the same manner with the 37-mer primer (5'-ATTGCGGTCCGTTCCTCCAGTCACGAC-CACCTTTTG-3') being hybridized to the ssM13mp9 DNA. The catedenated kDNA used for topoisomerase II assays was purchased from Topogen. The oligonucleotides used for preparation of triplex DNA were kindly supplied by Michael M. Seidman (Oncor Corp, Gaithersburg, MD).

**Triplex DNA was prepared using 5'-32P-labeled Y37 hybridized in a 1:1.5 ratio to R37 as duplex DNA.** The TC32 oligonucleotide was then bound to the 32P-PY37/R37 duplex by incubation overnight at 25 °C in triplex buffer (50 mM Tris-acetate, 5 mM Mg-acetate, pH 7.5, see Fig. 7).

**Resolvase Assays—Reactions were in 50 mM Tris-acetate, pH 7.8, 10% glycerol, 0.2 mM EDTA, 20 mM BME, 50 mM NaCl, 5 mM ATP, and 5 mM MgCl2, unless otherwise indicated.** The 5'-32P-labeled G4-DNA was incubated with the resolvase activity at 37 °C for 30 min unless otherwise indicated. Reactions containing crude extract were extracted with phenol/chloroform/isoamyl alcohol prior to addition of stop buffer. Other reactions were stopped by addition of stop buffer to a final concentration of 0.01% SDS, 15% glycerol, 0.01% xylene cyanol, 2-5 µl of each reaction was electrophoresed at 4 °C through a 12% nondenaturing polyacrylamide gel. Images were obtained and quantified on a Molecular Dynamics PhosphorImager. The percent G4-DNA resolved was calculated from (ssDNA)/(ssDNA + G4 DNA) × 100 with the background value from the negative control subtracted.

**Purification of Resolvase Activity—All steps were at 4 °C.** 260 g of placental tissue were cut into small pieces and homogenized in a Waring blender in 260 ml of 2 × buffer A (100 mM Tris-acetate, pH 7.8, 20% glycerol, 0.4 mM EDTA, 40 mM BME, 0.02% Triton X-100, 20 µg/ml leupeptin, 20 µg/ml pepstatin). The homogenate was spun at 3000 × g for 20 min, the supernatant was removed, and the pellet was washed with another 50 ml of buffer A plus 10 mM KPi, pH 7.8, and dialyzed against the same buffer (fraction I). The 96 ml of fraction I were mixed with 100 ml of hydroxylapatite resin, equilibrated in buffer A plus 10 mM KPi, pH 7.8, and stirred for 30 min. The resin was pelleted by spinning at 5000 × g for 20 min, the supernatant was removed, and the resin was washed with another 100 ml of buffer A plus 10 mM KPi, pH 7.8, in the same manner. Resolvase activity was eluted from the hydroxylapatite resin by stirring the resin in buffer A plus 0.5 mM KPi, pH 7.8, and pelleting the resin as described above. The supernatant was kept and dialyzed into buffer A in the presence of Chelex-100, yielding 138 ml of fraction II. Fraction II was loaded onto a 20-ml DEAE-Sepharose column that was equilibrated in buffer A. The column was eluted with a 100-ml linear gradient from 0 to 0.5 M NaCl in buffer A and then washed with another 50 ml of 0.5 M NaCl in buffer A. Resolvase activity eluted from the DEAE column in 2 peaks, an early broad peak and a second sharp peak that eluted at 0.5 M NaCl. Fractions in the second peak were pooled and dialyzed into buffer A plus 100 mM NaCl (fraction III). Fraction III was the first fraction that did not contain any detectable nuclease activity at the optimal MgCl2 concentration of 5 mM (see Fig. 1). One unit of activity is defined as the amount of activity that resolves 50% of 0.1 pmol G4-DNA as ssDNA in 30 min at 37 °C in a 30-µl resolvase reaction with 0.5 mM MgCl2. Since the crude extract, fraction I, and fraction II contain contaminating nuclease activity, the purification obtained can only be estimated at 100-fold. However, 6.5 ml of fraction III (1.2 units/µl, 1.17 mg/ml) were recovered and used for characterization of the resolvase activity.

**Kinetics of dNTPs Cofactors for Resolvase Activity—Resolvase reactions containing 1.2 units of activity, 0.1 pmol G4-DNA, 0.4 mM MgCl2, and increasing concentrations of ATP, GTP, and dATP were incubated at 37 °C for 30 min.** The percent G4-DNA resolved was determined as described above and plotted versus the concentration of NTP. The data were fit to a Michaelis-Menten curve by nonlinear regression using Kaleidagraph (AbelSoft software). The apparent Vmax and Km were determined for each nucleotide cofactor.

**Gel Filtration Chromatography—Gel filtration was done at 4 °C using a Superdex-200 HR column equilibrated in buffer A containing 0.5 M NaCl. 100 µl of protein standards (0.1 mg/ml ferritin (440 kDa), 1 mg/ml catalase (232 kDa), 1 mg/ml aldolase (158 kDa), 1 mg/ml bovine serum albumin (67 kDa), 1 mg/ml ovalbumin (43 kDa), and 0.1 mg/ml blue dextran) were injected into the column and the position of elution followed by A280. 100 µl of protein extract (concentrated from fraction III) containing 480 units of resolvase activity were loaded onto the column, and 0.25-ml fractions were collected and assayed for resolvase activity.

**RESULTS**

The ability of many chromosomal DNA sequences to form G4-DNA has led to the search for different cellular proteins that metabolize this high order DNA structure. We have identified a resolvase activity from human placenta tissue with the ability to resolve four-stranded G4-DNA to single-stranded DNA. The activity was identified by incubating protein extracts with G4-DNA in the presence of ATP and MgCl2. The resolvase activity was visualized on 12% non-denaturing polyacrylamide gels by following the conversion of the slow migrating 5'-32P-labeled four-stranded G4-DNA structure to a faster migrating form of DNA that is single-stranded DNA (ssPZ33). Titrating MgCl2 into resolvase assays with crude extracts showed that the four-stranded DNA (G4-DNA) becomes single-stranded (ssPZ33) as the MgCl2 concentration is increased from 0 to 0.2 mM (Fig. 1, lanes 10–14), but with higher concentrations of MgCl2 contaminating nuclease activity exists, resulting in a small product migrating slightly faster than the 5-mer marker DNA (Fig. 1, lanes 15–18). Marker DNAs were run in Fig. 1, lane 20. Fraction III was purified from...
The ability of helicases to unwind duplex DNA requires NTP(3,4). The optimal reaction temperature is in the range of 37–42 °C. It was shown that resolvase activity is sensitive to temperature and that maximal rate at 30 °C, 1.1% G4-DNA resolved/min, and was 2.3% resolved/min. The rate of the reaction was one-half of the rate of the reaction at 37 °C, 2.1% resolved/min, and at 55 °C = 0.16% resolved/min.

Any detectable nuclease activity (as described under “Experimental Procedures”) and has increasing resolvase activity with increasing concentrations of MgCl2 and maximal activity at 5 mM (Fig. 1, lanes 2–9). The resolvase activity is inhibited by EDTA and by proteinase K, indicating that it is dependent on the presence of protein and a divalent cation (results not shown). The resolvase activity has been partially purified using a 0–30% ammonium sulfate precipitation (fraction I), hydroxyapatite resin (fraction II), and DEAE chromatography (fraction III).

Resolvase Activity Is Sensitive to Temperature—To investigate whether the resolvase reaction may be a physiological catalytic reaction, the rate of the reaction was determined at five temperatures, 25, 30, 37, 42, and 55 °C (Fig. 2). At each of these temperatures, the rate of the reaction was linear for 25 min. The rate of the reaction at 37 °C was 2.1% G4-DNA resolved/min. At 42 °C, the reaction had a similar rate of 2.3% resolved/min. The rate of the reaction was one-half of the maximal rate at 30 °C, 1.1% G4-DNA resolved/min, and was considerably slower at both 25 °C and 55 °C. These data indicate that resolvase activity is sensitive to temperature and that the optimal reaction temperature is in the range of 37–42 °C.

Resolvase Activity Requires dNTP Hydrolysis for Activity—The ability of helicases to unwind duplex DNA requires NTP hydrolysis. We show that the resolvase reaction is dependent on the presence of a NTP (Fig. 3, lane 4), and incubation of fraction III with the nonhydrolyzable nucleotide analog, ATPγS, inhibits the resolvase reaction (Fig. 3, lane 3). With increased concentrations of ATPγS added to resolvase reactions with 5 mM ATP, a decrease in the amount of ssDNA product was seen (Fig. 3, lanes 5–10). With equal concentrations of ATP and ATPγS, a 50% inhibition of resolvase activity was seen (Fig. 3, lane 10). This indicates that the resolvase activity is dependent on NTP hydrolysis.

The apparent kinetics of the resolvase reaction with ATP, dATP, and GTP were examined to determine the cofactor preference of this activity (Table I). The apparent K m and V max values were determined by quantifying the reaction products at increasing concentrations of NTP. The percent G4-DNA resolved was plotted against the concentration of NTP, and the data were fit to the Michaelis-Menten equation. The apparent K m values for ATP and dATP were similar, 220 and 150 μM, but the V max was higher for GTP, 28%/30 min. These values give rate constants (V max/K m) that are a measure of the efficiency of the reaction in the presence of each cofactor. The resolvase activity is most efficient with ATP, 0.05%/30 min/μM, followed by dATP and then GTP, with rate constants of 0.023 and 0.015%/30 min/μM, respectively. A low amount of resolvase activity was seen with GTP, 0.02%/30 min/μM, whereas neither dCTP nor CTP could act as cofactors for the resolvase activity (data not shown).

Resolvase Products Are Unmodified ssDNA—There are at least two different ways by which the four-stranded G4 complexes can be separated into ssDNA. The resolvase activity may unwind the G4-DNA in an energy-dependent helicase-type mechanism or alternatively may act through a mechanism similar to DNA repair proteins altering the guanine base to release the Hoogsteen bonding. To further explore the mechanism of the resolvase reaction, products of the resolvase reactions were analyzed by chemical sequencing to determine if the DNA bases were modified during the reaction. The 32P-labeled resolved G4-DNA, intact G4-DNA, and ssPZ3 DNA were isolated from a 12% polyacrylamide gel and used in Maxam and Gilbert chemical sequencing reactions (25). In the G4-DNA the run of five guanosine residues that are involved in the Hoogsteen bonding were protected from methylation at N7 and were therefore not cleaved in the G reactions (Fig. 4, lane 2 and Ref.
15% urea-containing polyacrylamide gel. The nucleotide sequence of sequencing reactions. The cleavage products were then separated on a age with dimethylsulfate and piperidine in Maxam and Gilbert-type repair type of mechanism involving modification of the DNA.

Resolvase activity is a helicase-like activity and does not use a form of DNA (data not shown). These results imply that the ing protein shifting the equilibrium toward the single-stranded site (data not shown). Also, 1000-fold excess ssDNA did not inhibit the reaction, suggesting it is not simply a ssDNA bind-

Fig. 4. Resolvase products are unmodified single-stranded DNA. 5′-32P-labeled single-stranded PZ33 DNA (lane 1), G4-DNA (lane 2) and products of the resolvase reaction (lane 3) were isolated from a 12% nondenaturing polyacrylamide gel and subjected to chemical cleavage with dimethylsulfate and piperidine in Maxam and Gilbert-type sequencing reactions. The cleavage products were then separated on a 15% urea-containing polyacrylamide gel. The nucleotide sequence of PZ33 is labeled.

In contrast, the resolved G4-DNA sequenced identical to the ssPZ33 DNA, indicating that the N7 positions were no longer protected (Fig. 4, lanes 1 and 3). The bands generated from the sequencing reactions for both the ssPZ33 DNA and the resolved G4-DNA migrated identically through the denaturing polyacrylamide gel, indicating that the resolved DNA does not seem to be modified. The resolved G4-DNA was not cleaved by piperidine alone, indicating that it does not contain any abasic sites (data not shown). Also, 1000-fold excess ssDNA did not inhibit the reaction, suggesting it is not simply a ssDNA binding protein shifting the equilibrium toward the single-stranded form of DNA (data not shown). These results imply that the resolvase activity is a helicase-like activity and does not use a repair type of mechanism involving modification of the DNA.

Resolvase Activity Is a Novel Activity—A DNA unwinding reaction that is dependent on dNTP hydrolysis and a divalent cation has the potential to be a classical DNA helicase activity with a preferred substrate of duplex DNA. It has been shown that the dda protein of bacteriophage T4 can unwind duplex DNA (26), and SV40 T-antigen can unwind triplex DNA at pH 8.7 but not at pH 6.9 (27). Although, triplex DNA is a Hoogsteen-bonded structure similar in some aspects to G4-DNA, there have been no reports of these helicases unwinding G4-DNA. We tested the ability of the resolvase activity to unwind two different helicase substrates, an M13/17-mer, which contains a 17-nucleotide length of duplex DNA on ssM13 phage DNA, and an M13/37-mer, which again has 17 bases of duplex DNA but has 10 bases on both the 3′ and 5′ ends that are noncomplementary to the ssM13 template creating both 5′ and 3′ single-stranded tails. Using 1.2 units of resolvase activity (an amount that is within the linear range of the resolvase reaction), neither of the helicase substrates were unwound (Fig. 5, lanes 4, 7, and 10). Whereas, 1 μg of E. coli helicase II unwound both the M13/17-mer and M13/37-mer but not the G4-DNA (Fig. 5, lanes 5, 8, and 11). These data indicate resolvase is not a duplex DNA helicase activity but is an activity that prefers the G4-DNA structure.

Topoisomerase II is necessary for cellular DNA replication and maintenance of chromosomal DNA structure (28). Topoisomerase II acts in a sequence-specific manner and may be involved in chromosome segregation during meiosis (29). It has been shown that eukaryotic topoisomerase II cleaves G4-DNA and not duplex DNA containing the same nucleotide sequence (30). We addressed the question of whether resolvase activity separated the G4-DNA to ssDNA in a topoisomerase II catalyzed reaction. Topoisomerase II activity can be followed by its unique ability to decantenate kDNA. Resolvase activity was not able to decantenate kDNA (Fig. 6, lanes 5 and 8), whereas human topoisomerase II does decantenate the kDNA (Fig. 6, lane 4). This indicates that resolvase activity is not the product of topoisomerase II cleavage and ligation of the G4-DNA.

Because triplex DNA is also a form of Hoogsteen-bonded DNA that has been implicated in mutagenesis (14), the ability of the G4-DNA resolvase to unwind triplex DNA was tested (Fig. 7). Triplex DNA was made by incubating increasing concentrations of TC32 with the 32P labeled duplex DNA for 16 h at 25 °C (Fig. 7B, lanes 3–6). As an increasing concentration of the TC32 oligonucleotide is added to the binding reactions, an increase in the slowly migrating triplex form DNA is seen. The ability of the G4-DNA resolvase activity to unwind triplex DNA was tested by incubating 1.2 units of fraction III with triplex DNA at 37 °C for 30 min (Fig. 7C, lane 5). There is no detectable double- or single-stranded DNA formed upon incubation of triplex DNA with fraction III indicating that the resolvase activity does not unwind triplex DNA. These reactions were carried out with 100 nm triplex DNA in triplex binding buffer plus 5 mM MgCl2 and 5 mM ATP. Triplex DNA is not stable at 37 °C under the reaction conditions that give maximal resolvase activity (see resolvase assays under "Experimental Pro-
FIG. 6. Resolvase activity does not contain topoisomererase II activity. Topoisomererase II assays were done by incubating 0.25 μg of catenated kDNA with 0 and 1 units of human topoisomererase II (Topo II) (lanes 3 and 4) or 0, 0.6, 1.2, and 2.4 units of fraction III (lanes 5–8) at 37 °C for 30 min. Lane 1 contains linear DNA and lane 2 contains decatenated kDNA (nicked and relaxed). Products were run on a 1% agarose gel in the presence of 0.5 μg/ml ethidium bromide.

FIG. 7. Resolvase activity does not unwind triplex DNA. A, the sequence of oligonucleotides used to make triplex DNA. B, triplex DNA was made by binding 0, 50, 100, 200, and 400 nM TC32 to 100 nM 5'-32P-labeled Y37/R37 duplex, lanes 2–6, respectively. Lane 1 contains 100 nM 5'-32P-labeled Y37 prior to formation of duplex DNA. C, 100 nM triplex DNA was incubated at 37 °C for 30 min with 1.2 units of fraction III (lane 5); a control reaction without fraction III is also shown (lane 4). All triplex DNA experiments were visualized by running the samples on native 15% polyacrylamide gels in triplex buffer. Gels were 0.1 × 16 × 25 cm and were run at 4 °C.

FIG. 8. Gel filtration chromatography of resolvase activity. 11 mg of protein containing 480 units of activity pooled from a DEAE column was concentrated to a final volume of 100 μl and loaded onto a Superdex-200 HR column. 0.25-ml fractions were collected, and 1 μl of each fraction was assayed for resolvase activity (30-μl reactions with 0.2 pmols 5'-32P-labeled G4-DNA). Lanes 4–15 contain activity assays for fractions 45–56 with the elution of molecular mass markers catalase (232 kDa) and aldolase (158 kDa) shown. Lane 1 contains the negative control with no protein, lane 2 contains 2.4 units of fraction III, and lane 3 contains 0.1 μl of protein loaded onto the column. Reactions were incubated at 37 °C for 4 h, and products were separated on a 12% nondenaturing polyacrylamide gel.

We have identified a novel G4-DNA resolvase activity that resolves G4-DNA into ssDNA and have begun to address the mechanism by which the protein-dependent activity proceeds. The presence of many DNA sequences with the ability to form G4-DNA and the identification of different proteins that specifically interact with G4-DNA are support for the presence of G4-DNA and the identification of different proteins that specifically interact with G4-DNA. G4-DNA has been implicated in having effects on meiosis, recombination, telomere stability, and perhaps mutagenesis (6–9, 14–16, 32). The formation of G4-DNA may be advantageous for some cellular processes, for example meiosis. But in other instances, such as DNA replication and transcription, it has the potential to be problematic to the cell.

Chromosome separation may be a physiologic role of the G4-DNA resolvase. In both meiosis and telomere-telomere recombination, an interaction of four helices through quadruplex formation has been proposed to bring together guanine-rich DNA sequences (9). The SEP1/KEM1 protein cleaves the DNA on the 5' side of the G4 structure and may be needed for the chromatids to separate and move to the opposite poles during anaphase (17). The resolvase activity may also facilitate the separation of the guanine tetrad to allow for the cell cycle to proceed. Alternatively, telomeres keep their size by lengthening and shortening in small increments within upper and lower boundaries with the telomere length related to chromosome stability (34). However, telomerase cannot bind to G4-DNA.
structures in vitro (35). A G4-DNA metabolizing activity may be necessary to remove G4-DNA from the linear ends of the chromosomes and provide ssDNA that is substrate for the telomerase, thus allowing the length of the telomeres to be kept in equilibrium.

G4-DNA resolvase also may play a role in replication and/or transcription with the formation of G4-DNA under physiological conditions providing a potential block to the replication and/or transcription machinery. This is supported by both the stimulation of transcription-coupled repair by Hoogsteen-bonded triplex DNA and the identification of quadruplex DNA stimulation of transcription-coupled repair by Hoogsteen-

The activity is specific for the G4 structure as it does not unwind Hoogsteen-bonded triplex DNA and the identification of quadruplex DNA sequences with the ability to form G4-DNA that may need to be unwound by an activity like resolvase to allow the genome to maintain its integrity.

G4-DNA resolvase does not unwind duplex DNA substrates at a protein concentration that G4-DNA structures are unwound (Figs. 4 and 5), and the resolvase activity is not inhibited by 1000-fold excess single-stranded DNA (data not shown). These data indicate the resolvase activity seems to specifically recognize the Hoogsteen-bonded G4-DNA and separate the four strands of DNA in an energy-dependent helicase type of mechanism. The activity is specific for the G4 structure as it does not unwind Hoogsteen-bonded duplex DNA. It may also resolve G4-RNA, a structure that is formed in the dimerization domain of HIV RNA (2, 3). The existence of a G4-DNA resolvase in G4-RNA, a structure that is formed in the dimerization domain unwind Hoogsteen-bonded triplex DNA. It may also resolve

These data indicate the resolvase activity seems to specifically

Acknowledgments—We thank Eric Roesh (Wake Forest University Cancer Center) for synthesis of the oligonucleotides. Dr. Steven Matson (University of North Carolina) for the E. coli DNA helicase II, and Dr. Michael M. Seidman (OncoPharm, Gaithersburg, MD) for the oligonucleotides and procedures used to make the triplex DNA.

REFERENCES

1. Blackburn, E. H. (1991) Nature 350, 569–573
2. Awang, G., and Sen, G. (1993) Biochemistry 32, 11453–11457
3. Sundquist, W. I., and Heaphy, S. (1993) Proc. Natl. Acad. Sci. U. S.A. 90, 3393–3397
4. Shimizu, A., and Honjo, T. (1984) Cell 36, 891–893
5. Hammond-Kosack, M. C. U., Dobrinski, B., Lurz, R., and Docherty, K. (1992) Nucleic Acids Res. 20, 231–236
6. Sundquist, W. I., and Klug, A. (1989) Nature 342, 825–829
7. Sen, D., and Gilbert, W. (1990) Nature 344, 410–414
8. Williamson, J. R., Raghuraman, M. K., and Cech, T. R. (1989) Cell 59, 871–880
9. Sen, D., and Gilbert, W. (1988) Nature 334, 364–366
10. Kang, C., Zhang, X., Ratliff, R., Myzis, T., and Rich, A. (1992) Nature 356, 126–131
11. Smith, F. W., and Feigon, J. (1992) Biochemistry 31, 231–236
12. Wang, Y., and Patel, P. T. (1993) J. Mol. Biol. 234, 1171–1183
13. Lu, M., Guo, Q., and Kallenback, N. R. (1993) Biochemistry 32, 598–601
14. Wang, G., Seidman, M. M., and Glazer, P. M. (1996) Science 271, 862–865
15. Smith, S. S., Baker, D. A., and Jardines, L. A. (1989) Biochim. Biophys. Res. Commun. 160, 1397–1402
16. Akman, S. A., Lingeman, T. G., Doroshow, J. H., and Smith, S. S. (1991) Biochemistry 30, 8648–8653
17. Lu, Z., Frantz, J. D., Gilbert, W., and Tye, B.-K. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3157–3161
18. Weisman-Shomer, P., and Fry, M. (1993) J. Biol. Chem. 268, 3306–3312
19. Frantz, J. D., and Gilbert, W. (1995) J. Biol. Chem. 270, 9413–9419
20. Frantz, J. D., and Gilbert, W. (1995) J. Biol. Chem. 270, 20692–20697
21. Walsh, K., and Gualberto, A. (1992) J. Biol. Chem. 267, 13714–13718
22. Schierer, T., and Henderson, E. (1994) Biochemistry 33, 2240–2246
23. Lu, Z., and Gilbert, W. (1994) Cell 77, 1083–1092
24. Kim, J., Ljungdahl, P. O., and Fink, J. R. (1990) Genetics 126, 799–812
25. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
26. Maine, I. P., and Kodadek, T. (1994) Biochim. Biophys. Res. Commun. 204, 1119–1124
27. Peleg, M., Kopel, V., Borowiec, J. A., and Manor, H. (1995) Nucleic Acids Res. 23, 1292–1299
28. Kornberg, A., and Baker, T. A. (1992) DNA Replication, pp. 379–401, W. H. Freeman and Co., San Francisco
29. Rose, D., Thomas, W., and Holm, C. (1990) Cell 60, 1009–1017
30. Chung, I. K., Mehta, V. B., Spitzner, J. R., and Muller, M. T. (1992) Nucleic Acids Res. 20, 1973–1977
31. Weisman-Shomer, P., and Fry, M. (1994) Biochim. Biophys. Res. Commun. 205, 305–311
32. Giraldo, R., Suzuki, M., Chapman, L., and Rhodes, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7658–7662
33. Fang, G., and Cech, T. R. (1993) Cell 74, 875–885
34. Shampay, J., and Blackburn, E. H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 534–538
35. Fang, G., and Cech, T. R. (1993) Biochemistry 32, 11646–11657
36. Moraes, E. C., Keyse, S. M., and Tyrell, R. M. (1990) Carcinogenesis 11, 283–293