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Substrate-specific inhibition of RecQ helicase

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ABSTRACT

The RecQ helicases constitute a small but highly conserved helicase family. Proteins in this family are of particular interest because they are critical to maintenance of genomic stability in prokaryotes and eukaryotes. Eukaryotic RecQ helicase family members have been shown to unwind not only DNA duplexes but also DNAs with alternative structures, including structures stabilized by G quartets (G4 DNAs). We report that *Escherichia coli* RecQ can also unwind G4 DNAs, and that unwinding requires ATP and divalent cation. RecQ helicase is comparably active on duplex and G4 DNA substrates, as measured by direct comparison of protein activity and by competition assays. The porphyrin derivative, *N*-methyl mesoporphyrin IX (NMM), is a highly specific inhibitor of RecQ unwinding activity on G4 DNA but not duplex DNA: the inhibition constant (K_i) for NMM inhibition of G4 DNA unwinding is 1.7 μM, approximately two orders of magnitude below the K_i for inhibition of duplex DNA unwinding (>100 μM). NMM may therefore prove to be a valuable compound for substrate-specific inhibition of other RecQ family helicases *in vitro* and *in vivo*.

INTRODUCTION

DNA helicases of the RecQ family are highly conserved in prokaryotes and eukaryotes. This helicase family includes *Escherichia coli* RecQ (1), *Saccharomyces cerevisiae* Sgs1p (2), *Schizosaccharomyces pombe* Rqh1 (3), and at least five human homologs: BLM (4), WRN (5), RecQL (6), RecQ4 (7) and RecQ5 (7). The RecQ helicases share a central domain of ~400 amino acids, which contains an ATP binding site and seven helicase motifs, including the signature DEXH box. Long stretches of acidic residues characterize some of the eukaryotic enzymes, including Sgs1p, Rqh1, BLM and WRN. *Escherichia coli* RecQ, which is 610 residues in length, is the smallest member of this family, while Sgs1p, BLM and WRN are all >1400 amino acids in length.

RecQ family helicases are of great interest both because of their importance in maintaining genomic stability and also because human members of this helicase family are clearly associated with human genetic disease. Deficiencies in the human BLM helicase result in Bloom's syndrome, a disease characterized by growth retardation, immunodeficiency, impaired fertility, and a marked predisposition to a variety of malignancies; and, at the cellular level, dramatically elevated levels of sister chromatid exchange and formation of characteristic 'quadriradial' chromosomes (8,9). Deficiencies in WRN result in Werner's syndrome, which is associated with growth retardation, predisposition to development of a limited range of otherwise rare malignancies, and premature aging; and, at the cellular level, elevated levels of chromosomal deletions and translocations (10,11). A third human genetic disease associated with deficiencies in a RecQ family helicase is Rothmun–Thomson syndrome, characterized by early poikiloderma, skeletal abnormalities, juvenile cataracts and an elevated incidence of malignancies (12).

Because helicases in the RecQ family are highly conserved, single-celled organisms provide useful models for study of helicase properties and function. *Saccharomyces cerevisiae sgs1* mutants show increased rates of recombination, shortened life span and diminished fertility, as well as accumulation of extrachromosomal rDNA circles, which appear to correlate with aging (13–15). WRN and BLM can complement some phenotypes resulting from *sgs1* deficiency, showing that there is some conservation of function among the eukaryotic members of this helicase family (16,17).

Three domains within eukaryotic genomes are rich in the base guanine (G): the telomeres, the rDNA and, in mammals, the regions involved in immunoglobulin heavy chain switch recombination. *In vitro*, oligonucleotides carrying sequences from these regions spontaneously form structures stabilized by interactions between Gs. The basic unit of these structures is the G quartet (Fig. 1), a planar array of four Gs stabilized by hydrogen bonds (18). Stacking of planar and hydrophobic G quartets in turn produces higher order structures, which are extremely thermostable (19,20). These structures may be produced by intermolecular interactions between four parallel DNA strands, referred to as G4 DNA, or a combination of intra- and intermolecular interactions between two antiparallel strands, referred to as G2′ DNA (Fig. 1). One unusual property of both BLM (21) and Sgs1p (22) is the ability to unwind DNA containing G quartets, including both G4 DNA and G2′ DNA. In direct competition assays, BLM and Sgs1p helicases both demonstrate a 10–20-fold preference for G4 DNA compared to duplex DNA substrates. WRN can unwind alternative structures formed from fragile X syndrome d(CGG) consensus repeats, although it may not be active on G4 DNA structures

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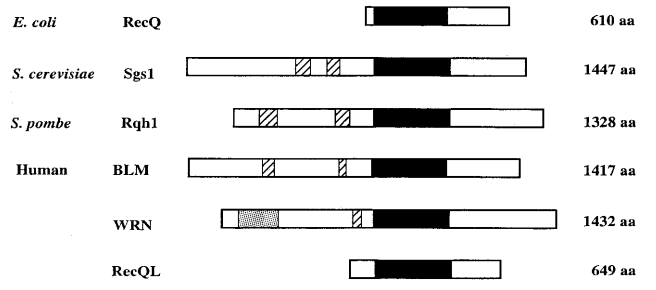

Figure 1. Schematic representation of members of the RecQ DNA helicase family. The proteins shown are *E.coli* RecQ, *S.cerevisiae* Sgs1p, *S.pombe* Rqh1 and human BLM, WRN and RecQL. The size of each protein is shown on the right. The conserved central helicase domain is shown as a black box; clusters of acidic residues are indicated by hatched boxes; and the exonuclease domain in WRN is indicated by a gray box.

formed by an immunoglobulin switch region sequence or by the vertebrate telomeric sequence, d(TTAGGG)$_n$ (23).

Because of the essential role of the RecQ family helicases in genomic stability, identification of inhibitors of helicase activity may have important implications for treatment of disease. Characterization of inhibitors of helicase activity can also be revealing about enzymatic mechanism. This has prompted us to search for inhibitors of RecQ family helicases, focusing on the highly conserved core of this helicase family represented by *E.coli* RecQ. *Escherichia coli* RecQ can unwind a broad range of DNA substrates, including duplex DNAs with blunt ends or 5′ or 3′ overhangs, forked DNA and three- or four-way junctions (1,24), but its unwinding activity on DNA substrates containing G quartets has not been tested. Here we report that *E.coli* RecQ can unwind G–G paired DNA substrates, and we identify two porphyrins that inhibit RecQ unwinding activity, meso-tetra (*N*-methyl-4-pyridyl) porphine tetra tosylate (T4) and *N*-methyl mesoporphyrin IX (NMM) (25). T4 is somewhat more inhibitory to unwinding of G–G paired DNA than duplex DNA; and NMM is a highly potent and specific inhibitor of unwinding of G–G paired DNA, but does not inhibit duplex DNA unwinding. The specificity and potency of NMM suggest that this compound may be valuable for analysis of RecQ helicase function *in vitro*, and it may also guide design of inhibitors for use *in vivo*.

MATERIALS AND METHODS

Overexpression and purification of *E.coli* RecQ

Plasmid pEG88 containing the complete *E.coli* RecQ cDNA cloned in the pQE31 expression vector (Qiagen) was a generous gift from Dr Charles Radding and Dr Ravi Gupta (Yale University, New Haven, CT). The plasmid was transformed into BL21(DE3) competent cells, which contain a plasmid pREP4 that expresses the *lacI*^{q} repressor. Cells were grown at 37°C in 18 l of LB broth (0.8% tryptone, 0.5% yeast extract, 0.5% NaCl) supplemented with ampicillin (100 µg/ml) and kanamycin (25 µg/ml). When the culture reached OD_{600} = 0.6, IPTG (1 mM) was added to induce RecQ expression, and, 3 h later, cells were harvested by centrifugation at 4°C and cell pellets were stored at –80°C until needed. To purify RecQ enzyme, cell pellets were thawed at room temperature and suspended in 80 ml cell lysis buffer (20 mM potassium phosphate pH 7.4, 20% sucrose, 500 mM KCl, 0.2 mM EDTA, 1 mM PMSF, 10 mM β-mercaptoethanol and 1 mg/ml lysozyme), incubated on ice for 20 min, sonicated, and centrifuged for 1 h at 14 000 *g* The supernatant was decanted and $(NH_4)_2SO_4$ was added slowly to 45% final saturation. After stirring for 30 min, pellets were collected by centrifugation for 40 min at 14 000 *g*, resuspended in 15 ml purification buffer (20 mM potassium phosphate pH 7.4, 10% glycerol, 50 mM KCl, 0.2 mM EDTA, 1 mM PMSF, 5 mM β-mercaptoethanol) and dialyzed overnight. The sample was then applied to a 13 ml SP-Sephadex column, washed with 60 ml purification buffer and fractionated with a 100 ml gradient of 50–750 mM KCl in purification buffer. Peak fractions (175–300 mM KCl) were determined by SDS–PAGE, pooled and dialyzed against 700 ml purification buffer for 1 h. The sample was loaded on a 6 ml Ni-NTA agarose column (Qiagen) which was washed with 20 ml purification buffer containing 20 mM imidazole, then eluted with 80 ml purification buffer containing a linear gradient of 50–100 mM imidazole. Peak fractions (50–75 mM imidazole) were pooled, dialyzed against 1 l storage buffer (20 mM Tris–HCl, 200 mM NaCl, 0.2 mM EDTA, 5 mM β-mercaptoethanol, 20% glycerol) for 3 h and stored at –80°C until used. Homogeneity of the resulting enzyme preparations was >95%, as determined by SDS–PAGE.

Formation of G4 and duplex DNA substrates

The following deoxyoligonucleotides were used: TP, TGGACCAGACCTAGCAGCTATGGGGGAGCTGGGGA-AGGTGGGAATGTGA; OX-1T, ACTGTCGTACTTGATA-TTTTGGGGTTTTGGGGAATGTGA; H1, GCATCGGCT-TCCCAACTAGCTTTTTTTTTT; K1, TTTTTTTTTTTTTT-TTTTTTTTTTTTTTTTGCTAGTTGGGAAGCCGATGC.

The TP sequence is a consensus repeat from the murine immunoglobulin Sγ2b switch region. OX-1T contains two copies of the Oxytricha telomeric repeat, d(T_4G_4). Duplex substrates were prepared by mixing equimolar ratios of H1 and K1 (final concentration of each oligonucleotide, 1.25 µM) in buffer containing 20 mM Tris–HCl pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT, heating at 94°C for 4 min and then cooling to room temperature during a 45 min incubation. Preparation of G–G paired DNAs was carried out as described (21). Briefly, gel-purified oligonucleotides were incubated at 60°C for 48 h at 2 µg/µl in TE (10 mM Tris–HCl pH 7.4, 1 mM EDTA) containing 1 M NaCl for G4 DNA formation or 1 M KCl for G2′ DNA formation. Samples were then resolved by electrophoresis on an 8% native polyacrylamide gel (29:1 acrylamide:bisacrylamide) in TBE containing 10 mM KCl, and bands corresponding to G4 DNA and G2′ DNA were visualized by UV shadowing, identified according to their relative mobilities, and excised. DNA was eluted from crushed gel slices by soaking at room temperature for 12 h in TE containing 50 mM NaCl and 25 mM KCl, precipitated with ethanol, washed and resuspended in TE containing 50 mM NaCl and 25 mM KCl. DNA was end-labeled with T4 polynucleotide kinase (New England Biolabs). G–G pairing was verified by assaying characteristic protection of the guanine N7 from methylation by dimethylsulfate (21). Assays contained G4 DNA substrate formed from the TP oligonucleotide, except where otherwise indicated.

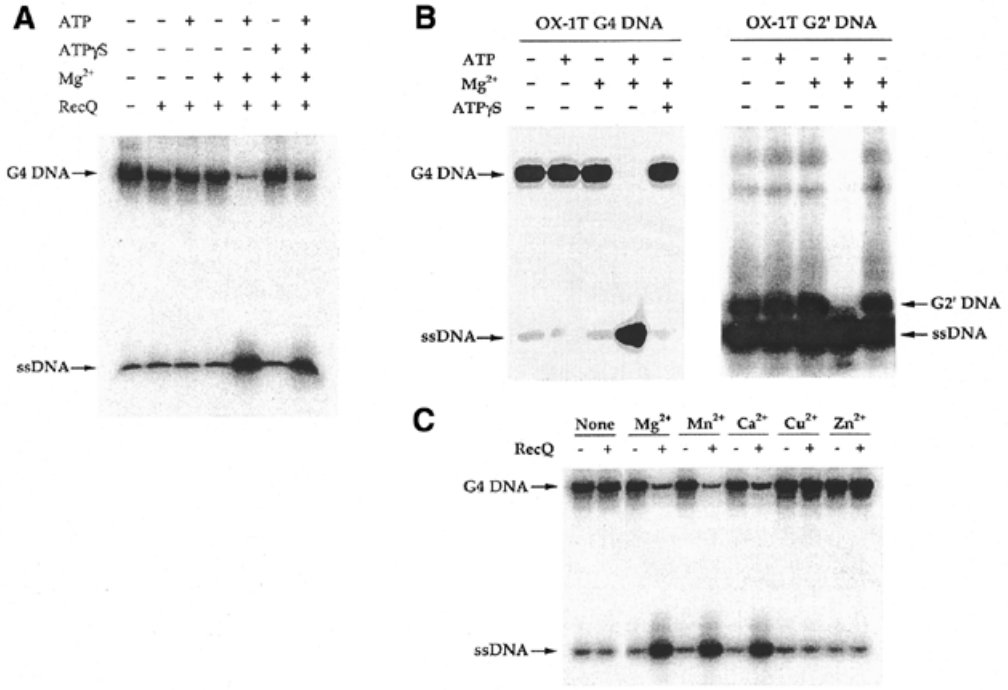

Figure 2. Unwinding of G4 DNA by *E.coli* RecQ DNA helicase. (**A**) ^{32}P-labeled G4 DNA (50 nM) formed from TP oligonucleotide was incubated with RecQ (50 nM) in the presence or absence of 2 mM ATP, 2 mM ATPγS and 2 mM Mg^{2+}, as indicated. (**B**) ^{32}P-labeled G4 DNA or G2′ DNA (5 nM) formed from $(T_4G_4)_2$, which carries two iterations of the Oxytricha telomeric repeat, was incubated with RecQ (50 nM) in the presence or absence of 2 mM ATP, 2 mM ATPγS and 2 mM Mg^{2+}, as indicated. (**C**) ^{32}P-labeled G4 DNA (50 nM) formed from the TP oligonucleotide was incubated with RecQ (50 nM) in the presence of 2 mM Mn^{2+}, Ca^{2+}, Cu^{2+} or Zn^{2+}, to replace Mg^{2+} in the reaction.

Helicase assays

Purified RecQ was incubated with radiolabeled DNA in 20 µl reactions in buffer containing 50 mM Tris–HCl pH 7.4, 2 mM $MgCl_2$, 2 mM ATP, 50 mM NaCl and 100 µg/ml BSA. The assay concentrations were 50 nM RecQ and 5 nM DNA, unless otherwise specified. Inhibitors tested were porphyrins T4 and NMM (Porphyrin Products, Inc., Logan, UT). Reactions were incubated at 37°C for 10 min and terminated by the addition of 5 µl 2.5% SDS, 0.1 M EDTA, to achieve final concentrations of 0.5% SDS and 20 mM EDTA. Samples were analyzed on 10% native polyacrylamide gels in 0.5× TBE, 10 mM KCl. Gels were dried and exposed for autoradiography or scanned and quantified by a phosphoimager.

RESULTS

Escherichia coli RecQ helicase unwinds G4 and G2′ DNA

The unwinding activity of purified *E.coli* RecQ on radiolabeled G4 DNA substrates was assayed in the presence and absence of Mg^{2+} and ATP. The G4 DNA substrate tested was formed from the TP oligonucleotide, which carries a consensus sequence from the murine immunoglobulin Sγ2b switch region. Figure 2A shows that unwinding of this substrate to produce single-stranded DNA products required the presence of both Mg^{2+} and ATP. Unwinding did not occur in the absence of divalent cation; nor if ATP was replaced by its non-hydrolyzable analog, ATPγS. BLM and Sgs1p similarly required Mg^{2+} and ATP to support G4 DNA unwinding activity (21,22).

ATPγS is a competitive inhibitor of BLM and Sgs1p unwinding activity; neither helicase was active in reactions containing both ATP and ATPγS (21,22). In contrast, although ATPγS did not support unwinding by RecQ, it did not inhibit unwinding in reactions that contained equimolar ATP (Fig. 2A, right lane). ATPγS is therefore not a potent competitive inhibitor of RecQ. The ATP binding site is within the conserved central helicase domain, and small sequence differences within this region in different RecQ family helicases might account for differential sensitivity to ATPγS.

G–G pairing can stabilize several distinctive DNA structures. Formation of these structures *in vitro* is influenced by the monovalent cations present (20). Typically, G-rich oligonucleotides form four-stranded structures (G4 DNA) in the presence of Na^+; and two-stranded hairpin dimers (G2′ DNA) in the presence of K^+. The four-stranded and two-stranded structures can be readily distinguished by mobility in native gels (Fig. 2B). We assayed RecQ helicase unwinding of G4 and G2′ structures formed from the OX-1T oligonucleotide, $(T_4G_4)_2$, which carries two iterations of the Oxytricha telomeric repeat (T_4G_4). Figure 2B shows that both of these structures are substrates for *E.coli* RecQ helicase. Unwinding required the presence of both Mg^{2+} and ATP, and was not supported by ATPγS.

No RecQ helicase activity was evident in the absence of divalent cations (Fig. 2C). Mg^{2+}, Mn^{2+} and Ca^{2+} supported unwinding activity, whereas Cu^{2+} and Zn^{2+} did not. The pattern of divalent cation dependence is similar to that observed in the ATPase activity assays of RecQ in the presence of single-stranded DNA (1).

RecQ is comparably active on G4 DNA and duplex DNA substrates

One intriguing property of both BLM and Sgs1p helicases is that they unwind G4 DNA more efficiently than standard Watson–Crick duplex DNA, as measured by direct comparison of activities or by competition experiments (21,22). To determine whether *E.coli* RecQ has a similar substrate preference, we compared unwinding of G4 DNA and duplex DNA by this enzyme. Because substrate preference of DNA helicases is determined in part by the structure of the DNA ends, the H1/K1 synthetic duplex 'fork' substrate was used in these assays. This substrate is designed to provide one blunt duplex end, one 3′ single-stranded tail and one 5′ single-stranded tail, and it is therefore a good substrate for a variety of helicases active on duplex DNA. Figure 3A compares RecQ helicase activity on G4 DNA and the H1/K1 duplex. Unwinding of both substrates was apparent at concentrations of RecQ as low as 10 nM and was complete in reactions carried out with 50 nM RecQ at 37°C for 10 min (Fig. 3A). There was no significant difference in the protein levels required for helicase activity on the two substrates in this direct assay. Although some duplex DNA is evident even in assays carried out at high levels of helicase for prolonged times, this is not unwound substrate, but duplex produced by spontaneous reannealing of complementary strands in the assay mix. Because the DNA concentrations used to measure unwinding are relatively low (5 nM) and incubation times short, G4 DNA does not spontaneously reform during the assay.

Substrate preference of *E.coli* RecQ was further examined in competition experiments. RecQ unwinding of radiolabeled G4 DNA was assayed in the presence of varing amounts of unlabeled H1/K1 duplex substrates, and, in parallel experiments, unwinding of H1/K1 duplex substrates was assayed in the presence of varying amounts of unlabeled G4 DNA (Fig. 3B). The percentage of DNA unwound in each set of reaction conditions was determined by phosphoimager analysis, and plotted (Fig. 3C). The competition curves were essentially identical, and RecQ therefore shows no preference for G4 DNA substrates relative to duplex DNA, or vice versa. This is a significant difference between *E.coli* RecQ and BLM (21) or Sgs1p (22), which are at least 10-fold more active on G4 DNA than on standard duplex substrates. This difference may reflect the fact that there are no characteristically G-rich domains in the *E.coli* genome.

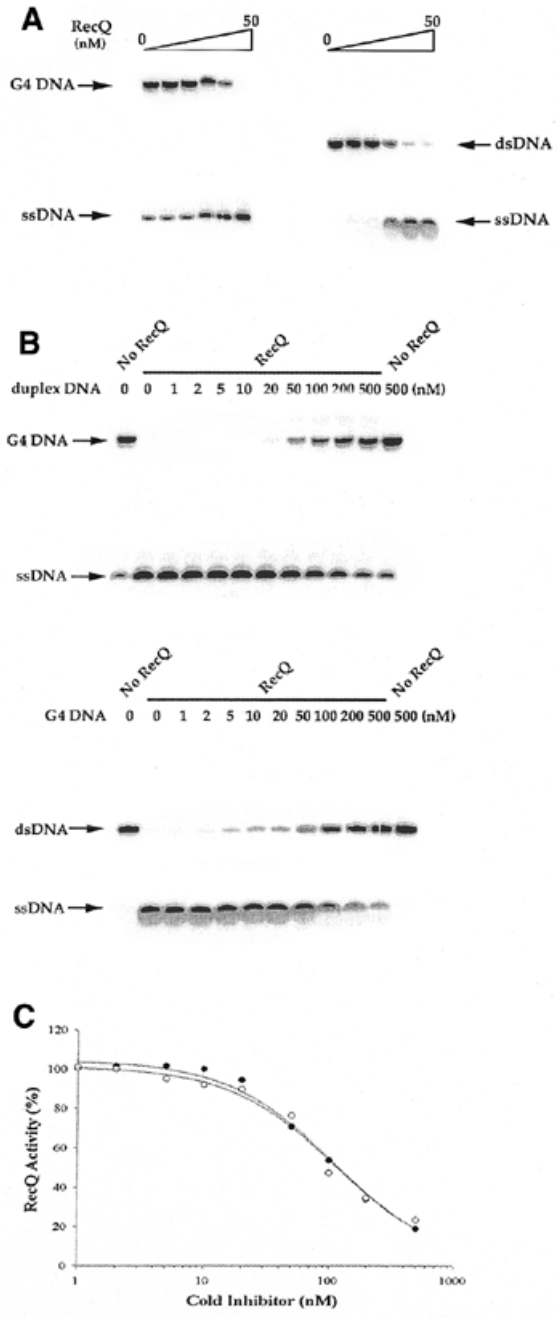

Figure 3. *E.coli* RecQ is comparably active on G4 DNA and duplex DNA. (**A**) Unwinding assay of *E.coli* RecQ on G4 and duplex DNA substrates. ^{32}P-labeled DNAs (5 nM) were incubated with RecQ at 0, 2, 5, 10 and 50 nM. (**B**) Competitive inhibition of unwinding. Top, ^{32}P-labeled G4 DNA (5 nM) was incubated with RecQ (50 nM) and indicated amounts of unlabeled duplex DNA. Bottom, ^{32}P-labeled duplex DNA (5 nM) was incubated with RecQ (50 nM) and indicated amounts of unlabeled G4 DNA competitor. (**C**) Inhibition of RecQ duplex and G4 DNA unwinding activity by competitor DNAs. Data from (B) were quantified by phosphoimager, and protein activity expressed as the fraction of labeled G4 DNA (filled circles) and duplex DNA (open circles) unwound during the course of the assay.

Substrate-specific inhibition of RecQ helicase activity by porphyrin derivatives

The porphyrin derivatives, T4 and NMM (Fig. 4A), have both been reported to interact with DNAs containing G–G paired regions, and NMM appears to bind preferentially to quadruplex relative to duplex DNAs (26–28). We therefore assayed inhibition of *E.coli* RecQ helicase unwinding of both duplex and G–G paired substrates by these two compounds. G4 DNA unwinding activity was inhibited at concentrations of T4 as low as 2 μM (Fig. 4B, left), while inhibition of duplex DNA unwinding was apparent only at T4 concentrations of 20 μM or greater (Fig. 4B, right). The presence of even low concentrations of T4 in an unwinding reaction retarded the mobility of both G4 and duplex DNA substrates (Fig. 4B). Retardation is likely to reflect interactions of T4 with DNAs.

NMM inhibited unwinding of G4 DNA at concentrations as low as 0.2 μM (Fig. 4C). In contrast, NMM did not inhibit unwinding of duplex DNA, even at concentrations as high as 100 μM (Fig. 4C). Unlike T4, which altered DNA mobility

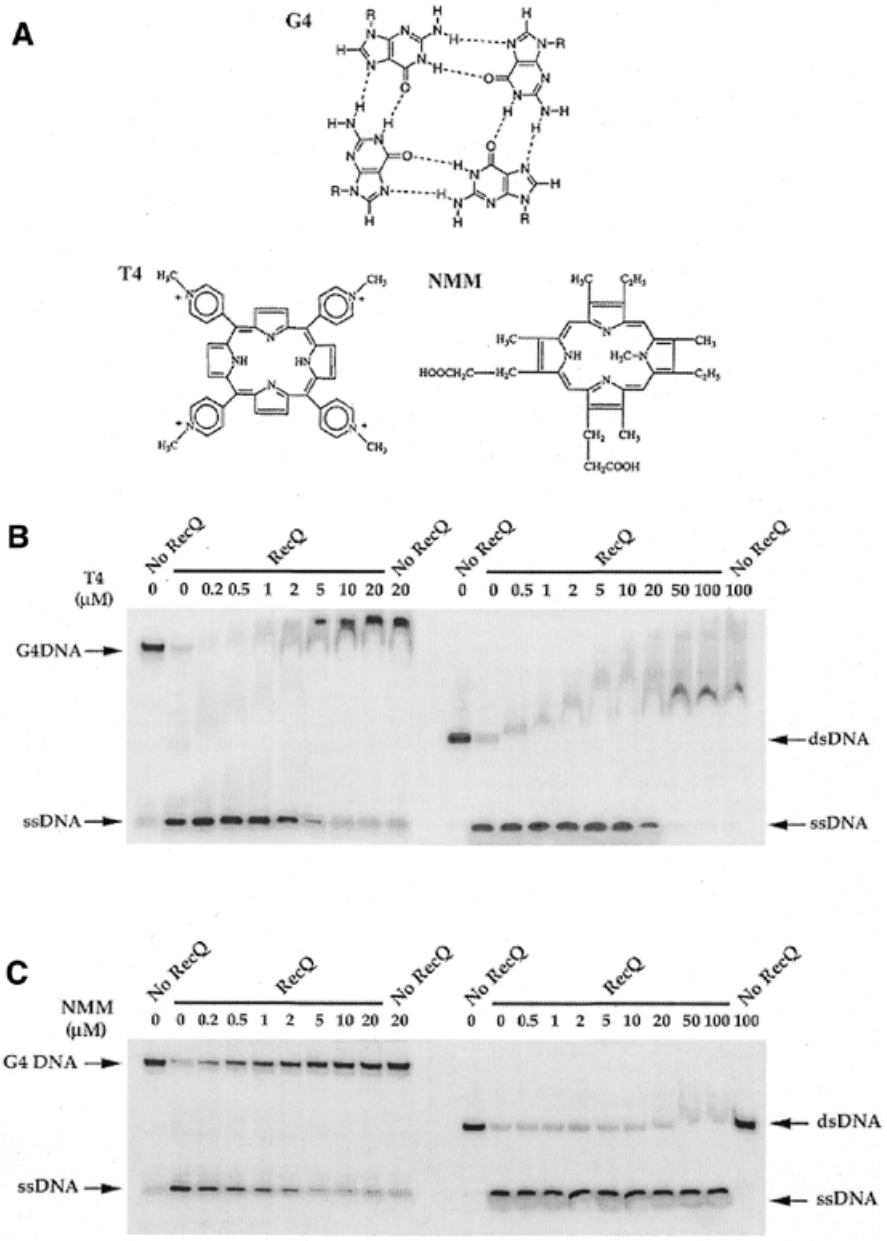

Figure 4. Porphyrins are potent inhibitors of *E.coli* RecQ helicase activity. (**A**) Schematic representation of the molecular structures of a G-quartet and porphyrin analogs T4 and NMM. (**B**) Inhibition of RecQ helicase activity by T4. Unwinding by RecQ (10 nm) was assayed on ^{32}P-labeled G4 DNA or duplex DNA substrates in the presence of the indicated amounts of T4. (**C**) Inhibition of RecQ helicase activity by NMM. Unwinding by RecQ (10 nm) was assayed on ^{32}P-labeled G4 DNA or duplex DNA substrates in the presence of the indicated amounts of NMM.

even when present at very low concentrations, NMM did not alter G4 DNA mobility even when this porphyrin was present at concentrations as high as 20 μM (Fig. 4C). The presence of NMM at concentrations >20 μM did cause a modest alteration in the mobility of duplex DNA.

K_i values for T4 and NMM were determined by graphing the data shown in Figure 4B and C, and are shown in Table 1. The K_i of T4 in reactions with G4 DNA substrates was 4.6 μM, while for duplex DNA substrates it was 21.8 μM. The K_i of NMM in reactions with G4 DNA substrates was 1.7 μM, while for duplex DNA substrates, K_i >100 μM. NMM is therefore a potent and substrate-specific inhibitor of G4 DNA unwinding activity.

DISCUSSION

Because of the importance of RecQ family helicases in maintenance of genomic stability, it is of considerable interest to identify inhibitors of these enzymes. We have shown that two porphyrin derivatives, T4 and NMM, inhibit RecQ helicase activity. The K_i for NMM inhibition of G4 DNA unwinding is 1.7 μM, approximately two orders of magnitude below the K_i for

Table 1. Substrate specificity of RecQ helicase inhibitors

Inhibitor	G4 DNA unwinding inhibition constant (μM)	Duplex DNA unwinding inhibition constant (μM)
T4	4.6	21.8
NMM	1.7	>100

Inhibition constants shown were determined as the concentration at which unwinding activity was inhibited by 50%. Values are means of two or more experiments.

inhibition of duplex DNA unwinding (>100 μM). The potency and specificity of NMM may make it a useful compound for inhibition of helicase activity on G–G paired substrates.

The molecular dimensions of the porphyrin ring are very similar to those of a planar quartet of four Gs (Fig. 4A), and a structure of T4 complexed with G2′ DNA has provided evidence for stacking of the porphyrin ring on a G quartet (29). A single-stranded 3′ tail is required for unwinding of G4 DNA by BLM and Sgs1p (21,22). It is likely that these helicases travel along the single-stranded tail until they reach the G–G paired region, where unwinding begins. This suggests that one mechanism by which porphyrin derivatives might inhibit helicase activity on G–G paired DNAs is by binding to DNA to prevent access of the helicase to the G–G paired regions. Nonetheless, porphyrins can interact tightly with specific proteins, as most classically exemplified by the globin–heme complex (30). While it is likely that inhibition reflects porphyrin–DNA interaction, we cannot exclude the possibility of inhibitor interaction with the enzyme.

NMM can bind selectively to G–G paired DNAs (26–28), and this property undoubtedly contributes to substrate-specific inhibition of unwinding. However, relative affinities of porphyrins for distinct DNA structures is unlikely to account entirely for the potency or specificity of inhibition by NMM. NMM inhibits G4 DNA unwinding somewhat better than does T4 (Table 1), but when binding of G–G paired DNAs by T4 and NMM was compared by equilibrium dialysis, T4 was shown to bind this nucleic acid species ~3-fold better than did NMM (28). Moreover, there is a 5-fold difference in inhibition constants for T4 inhibition of G4 DNA relative to duplex DNA unwinding (Table 1), but equilibrium dialysis found T4 showed little specificity for binding to G–G paired molecules relative to a variety of duplex substrates, including duplexes generated from synthetic homopolymers as well as duplexes isolated from natural sources (28). Further evidence that helicase inhibition by small molecules is not a simple reflection of affinity of those molecules for nucleic acid substrates comes from a recent survey, which tested the ability of a panel of reagents that interact with duplex DNA to inhibit WRN and BLM helicase activity (31). The most potent inhibitor identified was distamycin, which binds in the minor groove of duplex DNA. This compound inhibited unwinding of partial duplex substrates by BLM and WRN helicases with values of K_i in the 1 μM range. In contrast, K_i values of other compounds which bind in the minor groove of duplex DNA (DAPI, Hoechst 33258, netropsin) were ~10-fold higher. Moreover, despite its ability to inhibit duplex DNA unwinding by BLM and WRN (31), distamycin does not inhibit *E.coli* UvrD helicase (also known as helicase II), and it is a relatively poor inhibitor of *S.cerevisiae* Rad3 helicase (32).

RecQ shares the ability to unwind G–G paired substrates with human BLM and *S.cerevisiae* Sgs1p (21,22). The ability to unwind G–G paired DNAs is not common to all helicases: *E.coli* RecBCD is completely inactive on this substrate (21). However, while RecQ helicase (610 amino acids in length) is equally active on G–G paired and duplex DNA substrates, both full-length BLM and a truncated form of Sgs1p demonstrate preferential unwinding of G–G paired DNAs. The truncated Sgs1p polypeptide is about 869 residues in length (amino acids 400–1268), and contains both N- and C-terminal sequences that are not present in RecQ. The preferential unwinding activity of Sgs1p and BLM on G4 DNA substrates may therefore map to the helicase core itself, or to relatively short regions that flank the RecQ core homology region and are contained in truncated Sgs1p but not in RecQ. Mutational analysis and domain swap experiments should enable us to identify the regions of the eukaryotic polypeptides that contribute to preferential unwinding of G–G paired substrates.

The ability of eukaryotic RecQ family helicases to unwind G–G paired DNA provides a mechanism for removal of G–G paired structures, which may form within G-rich domains during transcription, replication or recombination, and which might otherwise interfere with DNA replication and normal cell division. The *E.coli* genome does not contain regions that are characteristically G-rich, like eukaryotic telomeres, rDNA or immunoglobulin heavy chain switch regions. *Escherichia coli* may therefore not require a helicase that is preferentially active on G–G paired substrates, providing a biological rationale for the fact that *E.coli* RecQ has comparable activity on duplex and G–G paired substrates. This raises a very interesting evolutionary question: was the ability to unwind G–G paired regions gained by the eukaryotic RecQ family enzymes, or lost by prokaryotic RecQ? Identification of the domains of the eukaryotic enzymes that contribute to preferential unwinding of G–G paired substrates may enable us to answer this question.

One critical aspect of drug design and discovery is identification of compounds that inhibit (or stimulate) specific processes while having minimal impact on other aspects of cell physiology. By virtue of both their essential biological functions and their unique chemistry, the G-rich domains of the mammalian genome would appear to be excellent targets for therapeutic agents. Porphyrin derivatives may display non-specific affinity for nucleic acids, and in many cases they are therefore not optimal inhibitors for use *in vitro* or *in vivo*. Nonetheless, the potency and substrate-specificity of NMM suggest that this compound, or its derivatives, may prove to be valuable reagents not only for biochemical studies but also for therapeutic purposes.

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