Importance of the Fourth Alpha-Helix within the CAP Homology Domain of Type II Topoisomerase for DNA Cleavage Site Recognition and Quinolone Action

Dirk Strumberg,1,4* John L. Nitiss,2,3 Jiaowang Dong,2 Jerrylaine Walker,2 Marc C. Nicklaus,3 Kurt W. Kohn,4 Jonathan G. Heddle,5‡ Anthony Maxwell,5‡ Siegfried Seebier,1 and Yves Pommier4

Laboratories of Molecular Pharmacology4 and Medical Chemistry,5 Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; Department of Internal Medicine and Medical Oncology, West German Cancer Center, University Medical School of Essen, 45122 Essen, Germany; Department of Molecular Pharmacology, St. Jude Children’s Research Hospital, Memphis, Tennessee 38105; and Department of Biochemistry, University of Leicester, Leicester LE1 7RH, United Kingdom

Received 9 January 2002/Returned for modification 12 April 2002/Accepted 22 May 2002

We report that point mutations causing alteration of the fourth alpha-helix (α4-helix) of the CAP homology domain of eukaryotic (Saccharomyces cerevisiae) type II topoisomerasers (Ser83Trp, Gln743Pro, and Thr744Pro) change the selection of type II topoisomerase-mediated DNA cleavage sites promoted by Ca2+ or produced by etoposide, the fluoroquinolone CP-115,953, or mitoxantrone. By contrast, Thr744Ala substitution had minimal effect on Ca2+ and drug-stimulated DNA cleavage sites, indicating the selectivity of single amino acid substitutions within the α4-helix on type II topoisomerase-mediated DNA cleavage. The equivalent mutation in the gene for Escherichia coli gyrases causing Ser83Trp also changed the DNA cleavage pattern generated by Ca2+ or quinolones. Finally, Thr744Pro substitution in the yeast type II topoisomerase rendered the enzyme sensitive to antibacterial quinolones. This study shows that the α4-helix within the conserved CAP homology domain of type II topoisomerases is critical for selecting the sites of DNA cleavage. It also demonstrates that selective amino acid residues in the α4-helix are important in determining the activity and possibly the binding of quinolones to the topoisomerase II-DNA complexes.

DNA topoisomerases are enzymes that catalyze changes in the topology of DNA via a mechanism involving the transient breakage and rejoicing of phosphodiester bonds in the DNA backbone (9, 59). Studies in both prokaryotic and eukaryotic cells have demonstrated the importance of topoisomerases in transcription, DNA replication, and chromosome segregation (41, 58). Type II topoisomerases (Top2p) are conserved among eukaryotes. Gyrase and topoisomerase IV are bacterial Top2 enzymes with significant sequence similarity to eukaryotic Top2 proteins (4, 39). Eukaryotic Top2 enzymes are homodimeric and cleave one DNA duplex (the G, or gate, segment) using two tyrosine residues (one from each Top2 monomer) to attack phosphodiester bonds on both DNA strands. In this process, each enzyme monomer becomes covalently attached to the 5′ end of the cleaved DNA by a phosphotyrosine linkage. Top2p then opens the DNA, bridging the gap in the G segment. A second DNA duplex (the T, or transported, segment) passes through the gap. Thereafter, the G segment is resealed, and the T segment is liberated from the enzyme (4, 53, 59).

Under physiological conditions, the covalent Top2p-DNA complexes (referred to as cleavage complexes) are normally short-lived intermediates in the catalytic cycle of the enzyme. Top2p requires the presence of a divalent cation for catalytic activity, which is Mg2+ and drug-stimulated DNA cleavage sites, indicating the selectivity of single amino acid substitutions within the α4-helix on type II topoisomerase-mediated DNA cleavage. The equivalent mutation in the gene for Escherichia coli gyrase causing Ser83Trp also changed the DNA cleavage pattern generated by Ca2+ or quinolones. Finally, Thr744Pro substitution in the yeast type II topoisomerase rendered the enzyme sensitive to antibacterial quinolones. This study shows that the α4-helix within the conserved CAP homology domain of type II topoisomerases is critical for selecting the sites of DNA cleavage. It also demonstrates that selective amino acid residues in the α4-helix are important in determining the activity and possibly the binding of quinolones to the topoisomerase II-DNA complexes.

* Corresponding author. Mailing address for Dirk Strumberg: Department of Internal Medicine (Cancer Research), University Medical School of Essen, Hufelandstr. 55, 45122 Essen, Germany. Phone: 49-(0)201-723-2027. Fax: 49-(0)201-723-5988. E-mail: dirk.strumberg@uni-essen.de. Mailing address for Yves Pommier: Laboratory of Molecular Pharmacology, Bldg. 37, Rm. 4E28, NIH, Bethesda, MD 20892-4255. Phone: (301) 496-5944. Fax: (301) 402-0752. E-mail: pommier@nih.gov.

‡ Present addresses: Protein Design Laboratory, Yokohama City University, Yokohama, Kanagawa 224-0045, Japan.

† Present address: Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom.
mitoxantrone, amsacrine, and ellipticines—and nonintercalators, whose main representatives are the demethylepipodophyllotoxins etoposide (VP-16), teniposide (VM-26), and the quinolones (37, 52), such as ciprofloxacin and norfloxacin, which are specific for bacterial Top2 enzymes (24, 36).

Previous studies showed that substitution of Gly747 to Glu of *Saccharomyces cerevisiae* Top2p (Fig. 1C) conferred resistance to doxorubicin, suggesting that the fourth alpha-helix (H92514-helix) is implicated in drug action (46). Furthermore, we recently reported that substitution of a conserved serine residue (Ser740Trp) in yeast Top2p confers resistance to quinolones and hypersensitivity to etoposide (26) and is associated with changes in DNA sequence preference at sites of cleavage (56). The first aim of the present study was to further investigate the role of the fourth helix (α4) within the CAP homology domain for DNA cleavage site recognition. CAP homology domains, i.e., regions that are similar to the DNA-binding domain of the *Escherichia coli* catabolite activator protein (23, 40), have been determined in both the crystal structure of a 92-kDa fragment of yeast Top2p (6) and a 59-kDa fragment of the gyrase A subunit (GyrA) of *E. coli* gyrase (39). We examined the effect of different single-amino-acid substitutions

---

**FIG. 1.** CAP homology domain and positions of Ser740, Gln743, and Thr744 in yeast Top2p. (A) Illustration of dimeric *S. cerevisiae* topoisomerase II structure (Protein Data Bank accession number 1bgw). Drawings were generated using the program QUANTA (version 97). The helical ribbon representation shows the 92-kDa fragment of the yeast enzyme with a DNA fragment modeled into each of the putative DNA-binding sites (6). The CAP homology domain of each protomer is highlighted in green. (B) Close view of the putative DNA-binding region, presenting the proposed proximity of the α4-helix within the CAP homology domain, including Ser740, Gln743, and Thr744 (in a stick model), to DNA. (C) Alignment of protein sequence for the yeast (Sc Top2p), human Top2p (Hu Top2p) and *E. coli* gyrase. The mutated residues studied in the present report are indicated by arrowheads. Shaded residues correspond to the α4-helix (5). No shading is shown for human Top2p because of lack of structural data. Boxed regions indicate similarity between the amino acid residues. Ser763 in the human Top2p sequence corresponds to Ser740 in yeast Top2p.
within the α4-helix (Fig. 1) on DNA cleavage patterns generated in the presence of Ca²⁺ or in the presence of nonintercalative and intercalative Top2p inhibitors. Four mutant yeast Top2p (Ser⁷⁴⁰Trp, Gln⁷⁴³Pro, Thr⁷⁴⁴Pro, and Thr⁷⁴⁴Ala) were compared to the wild-type enzyme. The Ser⁷⁴⁰Trp substitution in the GyrA subunit, which is equivalent to the Ser⁷⁴⁰Trp substitution in yeast Top2p (Fig. 1C), was also compared to wild-type gyrase. The second aim of this study was to examine the role of the α4-helix for drug activity to gain further insight in the interaction between Top2p inhibitors and the Top2p-DNA complexes. Together, our data demonstrate that specific amino acid residues within the α4-helix are critical for DNA sequence recognition with regard to cleavage of the G segment and for drug interaction within the enzyme-DNA complex. We also found that Thr⁷⁴⁴Pro substitution in yeast Top2p confers hypersensitivity to antibacterial quinolones.

MATERIALS AND METHODS

Materials, chemicals, and enzymes. Etoposide (VP16) was obtained from the Bristol-Myers Squibb Co., Wallingford, Conn. Mitoxantrone was from the Drug Synthesis and Chemistry Branch (National Cancer Institute, Bethesda, Md.). Azatoxin was provided by T. Macdonald, Department of Chemistry, University of Virginia, Charlottesville, CP-115,953 was a gift from P. R. McGuirk and T. D. Gootz of Pfizer Laboratories. Drug stock solutions were made in dimethyl sulfoxide at 10 mM. Further dilutions were made in distilled water immediately before use. Human c-myc inserted into pBR322, restriction enzymes, T4 polynucleotide kinase, polyacrylamide-bisacrylamide, and Taq DNA polymerase were
and the molecular dynamics simulations were carried out with QUANTA’s associated molecular mechanics program CHARMM, version 23.i, run on a separate computation host, a Digital 2100 AlphaServer 4/275, using the MSI parameter set version 22.0.

In order to generate a low-energy starting geometry for the subsequent molecular dynamics simulation, each of the wild-type and mutant structures was energy minimized in 5,000 steps of Adopted-Basis Newton-Raphson minimization. Each of these minimized structures was subjected to a total of 300 ps of molecular dynamics simulation using the Verlet algorithm for integration of the equations of motion. Using a step size of 1 fs, the structures were heated from 0 to 310 K in approximately 10 ps (9,892 steps), then equilibrated at 310 K for 40 (40,000 steps) with an equilibration frequency of 200 steps, and finally subjected to a simulation at 310 K for 250 ps (250,000 steps). Snapshot frames containing the current coordinates were written out every 50 steps.

RESULTS

Alteration of Ca\(^{2+}\)-promoted DNA cleavage by Ser\(^{740}\)Trp, Gln\(^{743}\)Pro, Thr\(^{744}\)Pro, and Thr\(^{744}\)Ala substitutions in yeast Top2p and by the Ser\(^{83}\)Trp substitution in bacterial GyrA. It is well known that DNA cleavage by type II DNA topoisomerases can be stimulated by a variety of drugs (2, 18, 33, 34, 37, 52), but cleavage can also be stimulated when Ca\(^{2+}\) is substituted for Mg\(^{2+}\) in the enzyme reaction (45, 50). In this case it is likely that the cleavage specificity reflects the natural sequence specificity of the enzyme. The Ca\(^{2+}\)-promoted, drug-independent DNA cleavage sites generated by yeast wild Type Top2p (Top2p\(^{WT}\)) and four yeast mutant proteins (Top2p\(^{S740W}\), Top2p\(^{G743A}\), Top2p\(^{P744A}\), and Top2p\(^{A744A}\)) were mapped in two different c-myc DNA fragments. Figure 2A and B show enzyme-specific differences. Cleavage was reduced at several sites for Top2p\(^{S740W}\) and Top2p\(^{G743A}\) compared to Top2p\(^{WT}\). For example, cleavage at positions 3124, 3147, 3156, 3226, 2706 or 2707, 2735, 2741, 2745, and 2754 was reduced for Top2p\(^{S740W}\) and Top2p\(^{G743A}\) compared to Top2p\(^{WT}\). For example, cleavage at positions 3124, 3147, 3156, 3226, 2706 or 2707, 2735, 2741, 2745, and 2754 was reduced for Top2p\(^{S740W}\) and Top2p\(^{G743A}\) compared to Top2p\(^{WT}\).

The GyrA\(^{S83W}\) substitution in E. coli gyrase is equivalent to Top2p\(^{S740W}\) in yeast (Fig. 1C) (26). Figure 2C shows a comparison of the Ca\(^{2+}\)-promoted DNA cleavage patterns for wild-type gyrase (gyrase\(^{WT}\)) and GyrA\(^{S83W}\). The number of Ca\(^{2+}\)-promoted DNA cleavage sites was changed for GyrA\(^{S83W}\) which is consistent with the corresponding results for Top2p\(^{S740W}\) (Fig. 2A and B). For GyrA\(^{S83W}\), cleavage at positions 3110, 3120, 3205, 3226, and 3230 was very low or not detectable. Conversely, cleavage was enhanced at positions 3150 and 3198. Differences in Ca\(^{2+}\)-promoted DNA cleavage patterns between GyrA\(^{S83W}\) and gyrase\(^{WT}\) were also observed in other c-myc fragments (data not shown). Together, these results demonstrate that single-amino-acid substitutions within the α-helix of the CAP homology domain affect Ca\(^{2+}\)-promoted DNA cleavage for both Top2p and gyrase.
Differences in DNA cleavage patterns generated by yeast Top2p $^{S740W}$, Top2p $^{Q743P}$, Top2p $^{T744P}$, Top2p $^{T744A}$, and Top2p WT in the presence of Top2p poisons. To further investigate differences between Top2p $^{S740W}$, Top2p $^{Q743P}$, Top2p $^{T744P}$, Top2p $^{T744A}$, and Top2p WT, we compared drug-stimulated DNA cleavage patterns. Etoposide results are shown in Fig. 3A. Results for the fluoroquinolone CP-115,953 are shown in Fig. 3B, and those for mitoxantrone are shown in Fig. 3C.

Top2p $^{S740W}$ (as well as the corresponding yeast mutant) has been previously characterized as being resistant to the fluoroquinolone CP-115,953 and hypersensitive to etoposide (26, 56). Compared with Top2p WT and the other mutant proteins, several cleavage sites stimulated by etoposide were markedly enhanced with Top2p $^{S740W}$, e.g., at positions 2711 to 2712, 2781, 2983, 3011, 3019, and 3026 (Fig. 3A).

Proline substitutions at positions 744 and 743 also changed the distribution of Top2p-mediated DNA cleavages. These effects were more pronounced for Top2p $^{T744P}$ than for Top2p $^{Q743P}$ (Fig. 3). By contrast, Top2p $^{T744A}$ behaved very similarly to Top2p WT (Fig. 3).

Together, the data shown in Fig. 3 demonstrate that single mutations in the $\alpha$-helix of Top2p ($^{S740}$Trp, $^{Gln743}$Pro, and $^{Thr744}$Pro) changed enzyme-specific DNA cleavage patterns produced by nonintercalating and intercalating drugs.

Differences in quinolone-generated DNA cleavage patterns by the mutant GyrA $^{S83W}$ and wild-type DNA gyrase. We next tested the role of the corresponding $\alpha$-helix in the bacterial topoisomerase II, DNA gyrase. Figure 4 shows a comparison of the DNA cleavage patterns observed in the presence of two different quinolones, CP-115,953 and ciprofloxacin. Because the GyrA $^{S83W}$ mutant is quinolone resistant (13, 42, 58, 60) and therefore bound to give less cleavage than gyrase WT, we chose drug concentrations (100 $\mu$M) that are approximately three times the 50% inhibitory concentration for supercoiling (data not shown). As for yeast Top2p, the S83W substitution
changed the enzyme cleavage pattern. Several cleavage sites were reduced in intensity for GyrA S83W in the presence of CP-115,953 and ciprofloxacin (e.g., 2747, 2744, 2772, 2770, 2991, 3020, 3017, and 3020), whereas other sites were enhanced (e.g., 2722, 2893, 2768, 2794, 2805, and 2962). These results demonstrate that the GyrAS83W mutation markedly affects the DNA sequence specificity of DNA gyrase.

Comparison of yeast Top2p T744P and E. coli gyrase DNA cleavage sites generated in the presence of quinolones. In contrast to the mutation causing Ser740Trp, which confers quinolone resistance, the Thr744Pro substitution causes hyper-sensitivity to CP-115,953 (14) (Fig. 3). Figure 5 compares DNA cleavage produced by CP-115,953, ciprofloxacin, and norfloxacin for GyrA WT, Top2p WT, and Top2p T744P. Remarkably, prominent cleavage was observed for Top2p T744P in the presence of ciprofloxacin and norfloxacin, whose antibacterial ac-

FIG. 3. DNA cleavage patterns generated by yeast Top2p WT, Top2p S740W, Top2p Q743P, Top2p T744P, and Top2p T744A enzymes in the presence of etoposide, the fluoroquinolone CP-115,953, and the intercalator mitoxantrone. DNA fragments from the junction between the c-myc first intron and first exon between positions 2671 and 3072 were prepared by PCR using one primer labeled with 32P at the 5’ terminus. The left-hand panels show labeling of the upper DNA strand at position 2671. The right-hand panels show that labeling of the lower DNA strand was at position 3072. Top2 enzymes are indicated above each lane. Lanes: y WT, yeast wild-type Top2p; y S740W, yeast Top2p S740W; y Q743P, yeast Top2p Q743P; y T744P, yeast Top2p T744P; y T744A, yeast Top2p T744A. Concentrations were 100 μM for etoposide (A) and CP-115,953 (B) and 1 μM for mitoxantrone (C). Reactions were performed at 37°C for 30 min in the presence of 5 mM MgCl2. Purine ladders were obtained after formic acid reaction. Numbers correspond to genomic positions of the nucleotide covalently linked to Top2p via the 5’ phosphate. Double-headed arrows correspond to DNA cleavage sites with a 4-bp stagger that represent potential DNA double-strand breaks.
Activities are due to specific inhibition of bacterial Top2p and have no effect on mammalian Top2p.

A number of cleavage sites stimulated by Top2p<sup>T744P</sup> in the presence of quinolones matched the <i>E. coli</i> DNA gyrase sites (e.g., at positions 3202, 3179, 3145, 3121, 3108, 3066, and 3111). However, other sites were different for DNA gyrase. Enhanced sensitivity to antibacterial quinolones by the Thr<sup>744</sup>Pro substitution in yeast Top2p was also detected in other DNA fragments (data not shown).

Base preference of the CP-115,953-stabilized cleavage complexes for mutant Top2p<sup>T744P</sup> enzyme. Because we observed that the Thr<sup>744</sup>Pro substitution enhanced sensitivity to quinolones (including the fluoroquinolone CP-115,953) and produced common DNA cleavage sites with <i>E. coli</i> DNA gyrase, we examined whether this particular amino acid substitution affected the DNA base preference of yeast Top2p in the presence of CP-115,953. Cleavage sites for the three c-myc DNA fragments and one c-jun fragment (see Materials and Methods) (Fig. 6) were analyzed for both DNA strands. For the wild-type yeast Top2p, CP-115,953 preferentially stabilized sites with C or T at position −1 (C<sup>−1</sup> + T<sup>−1</sup>: 49 out of 65 sites) and A or G at position +1 (A<sup>+1</sup> + G<sup>+1</sup>: 54 out of 65 sites). Complementary preferences (although slightly weaker) were seen for T<sup>+4</sup> and G<sup>+5</sup>.

Top2p<sup>T744P</sup> also demonstrated a preference for T at position −1, which was higher than that for the wild-type enzyme (41 out of 89 sites), in combination with preference for G at position +1 (44 out of 89 sites). However, preferences for C at position −1 and A at position +1 were not detectable in the mutant protein (Thr<sup>744</sup>Pro). Moreover, complementary preference also changed to A<sup>+5</sup>. Because bacterial Top2 enzymes are also sensitive to CP-115,953 (Fig. 5), we analyzed the base preferences for DNA gyrase. The observed preferences for T at position −1 (41 out of 107 sites) and G at position +1 (50

FIG. 3—Continued.
out of 107 sites) are in agreement with previous reports obtained with the antibacterial fluoroquinolones (12, 17, 21). These data indicate that enhanced quinolone sensitivity of the mutation causing Thr744Pro is associated with a shift in base sequence preferences, resembling that of DNA gyrase.

**DISCUSSION**

The A' domains of eukaryotic Top2 enzymes (homologous to the amino terminus of the A subunit of gyrase) comprise the primary DNA binding sites and include the catalytic tyrosine...
Y782 for the yeast *S. cerevisiae* (59), which is homologous to Y122 for *E. coli* gyrase (6, 25, 39). DNA footprinting analyses indicate that for eukaryotic Top2p and bacterial topoisomerase IV, ~15 to 35 bp of DNA is tightly bound to the enzyme (30, 47). In the case of gyrase, a more extensive stretch of DNA (~130 bp) is protected by the enzyme (43). However, the central section of this region around the DNA cleavage site is very similar in size to that bound by Top2p and topoisomerase IV (12, 21, 39). The length of this DNA segment is consistent with the combined length of the A’ grooves present in the Top2p and GyrA structures (6, 39). Within the A’ domain of Top2p, a 29-kDa fragment containing the active-site tyrosine and the helix-turn-helix motif from the CAP homology domain can be cross-linked to DNA (27), and protein footprinting suggest that the binding of DNA protects lysines from chemical modification within the same region (31). CAP homology domains contain typically a three-alpha-helix bundle backed by beta-sheets. Two adjacent alpha-helices, usually the second helix (referred to as alpha3-helix in yeast Top2p according to reference 6) and the third helix (referred to as alpha4-helix of the bundle in yeast Top2p according to reference 6), are connected by a short turn. This motif known as the helix-turn-helix is responsible for many of the critical contacts between CAP-like proteins and DNA. The second helix of this motif generally inserts into the major groove of DNA with the turn contacting the phosphodiester backbone (5, 23, 40) (Fig. 1).

Our observations suggest that specific substitutions in the alpha4-helix (Thr744Pro, Gln743Pro, and Ser740Trp of eukaryotic Top2p), which corresponds to the third helix of the CAP homology domain, can change the enzyme-DNA interactions, as reflected by differences in DNA cleavage patterns induced in the presence of Ca2+ or in the presence of intercalating and nonintercalating drugs. We also observed differences in base sequence preferences. We interpret these results as an indication that the alpha4-helix of the Top2p CAP homology domain is critical for DNA sequence recognition prior to cleavage of the DNA. 

FIG. 5. Generation of DNA cleavage by the yeast Top2pT744P mutant in the presence of antibacterial quinolones. A 254-bp DNA fragment from the c-myc first intron was prepared. (A) Labeling of the upper DNA strand at position 3035. (B) Labeling of the lower DNA strand at position 3288. Drugs (100 μM each) and enzymes are indicated above the lanes. Lanes: yWT, yeast wild-type Top2p; Y T744P, Top2pT744P. Numbers correspond to genomic positions of the nucleotide covalently linked to Top 2p. Double-headed arrows correspond to DNA cleavage sites with a 4-bp stagger that represent potential DNA double-strand breaks.
FIG. 6. Probability of the observed base frequency deviations at Top2p cleavage sites for the yeast wild-type enzyme, for Top2p T744P and for E. coli gyrase in the presence of CP-115,953. Position 0 corresponds to the cleavage site, and positions −1 and +1 to the bases immediately 3’ and 5’ from the cleavage site, respectively. The panels present the probability of the observed base frequency deviations from expectation for the indicated enzyme. On the y axis, $P$ is the probability of observing that deviation or more, either as excess (above baseline) or deficiency (below baseline), relative to the expected frequency of each individual base (48). Cleavage sites for the yeast wild-type Top2p (A), the yeast Top2p T744P (B), and E. coli gyrase (C) were analyzed after treatment with 100 μM CP-115,953.
G strand, which is consistent with the proposed binding of DNA to yeast Top2p with the α4-helix close to the catalytic tyrosine and to the ends of the cleaved DNA (6). Figure 1 presents the positions of Ser740, Gln743, and Thr744 in the yeast Top2 protein (4, 6). These conserved residues (Fig. 1C) tend to be buried inside the protein, while the nonconserved residues Ser740 and Gln743 are more solvent accessible (22) (Fig. 1B). The nonconserved amino acid residues might contribute to the observed differences in DNA sequence selectivity and drug sensitivity between the bacterial, the yeast, and the human Top2 enzymes (22, 55).

Other regions of Top2p must interact with different DNA segments, such as the T segment for the strand passage reaction. The B' domain (homologous to B subunit of gyrase) is also essential for DNA binding and cleavage (7, 19, 20, 31, 35). Recently it was proposed that DNA breakage and rejoining by Top2p involve the coordinated action of the CAP homology domain in the A' fragment containing the active-site tyrosine together with the Rossmann fold in the B' fragment (5, 16, 35; J. G. Heddle, and A. Maxwell, submitted for publication). The Rossmann fold, which is important for DNA binding and cleavage reactions, contains a number of highly conserved acidic residues that probably bind divalent metals (5, 16, 35). Structural and biochemical studies suggest that the active-site tyrosine in the DNA-binding domain of one protomer cooperates with several residues in the Rossmann fold of the other protomer (16, 35). Our data suggest that not only the active-site tyrosine and the Rossmann fold domain but also the α4-helix of the CAP domain are involved in the concerted molecular actions, including DNA interactions leading to sequence-specific cleavage of the G-strand duplex DNA.

We have shown that the Ca2+- and drug-generated DNA cleavage patterns of Top2pT744P and Top2pG743P are different from those of Top2pT744A and Top2pWT and that Top2pT744A is comparable to Top2pWT. Since the Top2pT744P substitution does not lead to enhanced drug sensitivity (14), the change in the observed pattern of Top2p cleavage sites cannot arise solely from global quantitative alterations. Also, since Top2pT744A does not have a pattern of cleavage that differs from the wild-type enzyme, Thr744 probably does not play a crucial role in determining cleavage site selection by the enzyme. Since detailed structural data for any of the mutant proteins are not yet available, we modeled the specific molecular changes that might result from the Thr744Ala/Pro and Gln743Pro substitutions in the yeast Top2p structure (6). Molecular dynamics simulations of the Thr744Pro mutant Top2p predicted structural changes within the CAP homology domain (data not shown; see also reference 14). Compared to wild-type Top2p and Top2pT744A, the Thr744Pro substitution was associated with a kink in the α4-helix and a bending angle of approximately 37°. The helical structure was preserved on both sides of the kink (data not shown). These simulations suggest that an alteration in the secondary structure of the α4-helix can alter protein-DNA interactions. It is important to note, however, that changes in other parts of the protein also occurred. Therefore, it is premature to conclude that all of the biochemical alterations observed in the mutant proteins arise solely from changes in the α4-helix.

A key issue in understanding the mechanism of action of Top2p targeting drugs is the determination of where the drugs bind in the Top2p/DNA complex. Our present findings indicate that the same Top2p domain that presumably interacts with DNA is also critical for drug interaction in the Top2p/DNA/drug ternary complex. In particular, the solvent-exposed residues Ser740, Gln743, and to a lesser extent Thr744, which affect drug activity, are important, directly or indirectly, for drug binding to the Top2p/DNA complex (22).

An intriguing finding arising from this work is that a quinolone-hypersensitive yeast Top2p mutant, resulting in Thr744Pro substitution, changed the DNA cleavage pattern in the presence of quinolones so that it more closely resembled the DNA cleavage pattern of a quinolone-resistant prokaryotic topoisomerase II. Quinolones act against eukaryotic topoisomerase II by accelerating the rate of DNA cleavage (51). Recent studies have shown that along with gyrase and topoisomerase IV of E. coli, quinolones can also inhibit religation (1, 28). A plausible model is that the quinolone-hypersensitive mutant allows quinolones to inhibit religation as well. By this hypothesis, the action of quinolones against Top2pThr744Pro more closely resembles the action of quinolones against prokaryotic topoisomerase II, demonstrating conservation of mechanisms of drug action between eukaryotic and prokaryotic type II topoisomerases. Thus, the quinolone-protein interaction domains between the prokaryotic and eukaryotic enzymes may be comparable, with only limited differences preventing antibacterial quinolones from acting against eukaryotic Top2p.

ACKNOWLEDGMENTS

Dirk Strumberg was supported by the Deutsche Forschungsgemeinschaft (grant Str 527/2-1), Bonn, Germany. John L. Nitiss was supported by grants from the National Cancer Institute (CA52814 and CA21765) and the American Lebanese Syrian Associated Charities (ALSAC). Jonathan G. Heddle was supported by the Medical Research Council (United Kingdom).

REFERENCES


demic Press, New York, N.Y.
