Differential Poisoning of Human and *Aspergillus nidulans* DNA Topoisomerase I by Bi- and Terbenzimidazoles†

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ABSTRACT: DNA topoisomerase I has been partially purified from *Aspergillus nidulans*. The purified enzyme is most likely the major nuclear DNA topoisomerase I on the basis of the following findings. (1) Purified DNA topoisomerase I can relax both positively and negatively supercoiled DNA. (2) Neither an energy cofactor nor Mg(II) is required for the relaxation or the cleavage reaction of the enzyme. On the basis of a phosphate-transfer experiment, the *Aspergillus* topoisomerase I was shown to have a molecular mass (M_r) of 105 kDa. The differential sensitivity of the human and *Aspergillus* topoisomerase I was compared using a number of known human DNA topoisomerase I poisons. Like human DNA topoisomerase I, *Aspergillus* topoisomerase I is highly sensitive to the poisoning activity of camptothecin and a number of bi- and terbenzimidazoles. However, unlike human topoisomerase I, *Aspergillus* topoisomerase I is completely resistant to monobenzimidazoles, protoberberines (e.g. coralyne), and nitidine. Cytotoxicity studies using yeast expressing human and yeast topoisomerase I cDNAs have also demonstrated a similar differential sensitivity of yeast topoisomerase I to these human topoisomerase I poisons. These results together suggest that the nuclear fungal topoisomerase I may be sufficiently different from its human counterpart to serve as a molecular target for the development of antifungal drugs.

DNA topoisomerases have been shown to be important new molecular targets for a broad range of therapeutics such as antimicrobials, antiparasitics, and antitumor drugs. By interfering with the breakage/reunion reaction of DNA topoisomerases, many drugs have been shown to convert these important nuclear enzymes into DNA-breaking nucleases, resulting in efficient cell killing (Liu, 1994; Wang, 1996).

While type II DNA topoisomerases have been recognized as important molecular targets for antibiotics (e.g. quinolones) (Neu, 1994) and many antitumor drugs (e.g. etoposide, doxorubicin, and mitoxantrone) (Liu, 1989), type I DNA topoisomerases have not until recently been recognized as equally important molecular targets for therapeutics. The recognition of human DNA topoisomerase I as a molecular target for the anticancer drug camptothecin has stimulated searches for new topoisomerase I-targeting drugs (Hsiang *et al.*, 1985; Andoh *et al.*, 1987; Nitiss & Wang, 1988; Bjornsti *et al.*, 1989; Pommier, 1996). To date, many new topoisomerase I-poisoning compounds have been identified, including nitidine, fagaronine, protoberberines, bulgarein, saintopin, intoplicin, indocarbazole, and mono-, bi-, and terbenzimidazoles (Yamashita *et al.*, 1991; Poddevin *et al.*, 1993; Janin *et al.*, 1993; Leteurtre *et al.*, 1994; Wang *et al.*, 1993; Chen *et al.*, 1993; Kim *et al.*, 1996a,b; Makhey *et al.*, 1995, 1996; Gatto *et al.*, 1996). All these compounds, like camptothecin, interfere with the breakage/reunion reaction of topoisomerase I, resulting in accumulation of the covalent intermediate, in which topoisomerase I is reversibly trapped in a cleaved state, termed the cleavable complex (Hsiang *et al.*, 1985; Porter & Champoux, 1989; Jaxel *et al.*, 1991).

Mycotic infections have become increasingly important in the last two decades, causing high mortality among immunocompromised patients, such as transplant recipients and cancer and AIDS patients. The expanding patient population and some existing problems in current antifungal chemotherapy demand more effective and safe antifungal agents for the treatment of this increasingly important class of opportunistic infections. On the basis of studies in *Saccharomyces cerevisiae* and *Candida albicans*, nuclear fungal topoisomerase I shows promise for being a new molecular target for antifungals [Shen *et al.*, 1992; Fostel & Montgomery, 1995; Fostel *et al.*, 1992; for reviews, see Shen and Fostel (1994) and Nitiss (1994)]. Studies in *S. cerevisiae* have established topoisomerase I to be a fungicidal target for camptothecin (Nitiss & Wang, 1988). Studies in *C. albicans* have demonstrated differences in the sensitivity of the human and *Candida* topoisomerase I as aminocatechol A-3253 (Fostel & Montgomery, 1995).

Aspergillus fumigatus and *Aspergillus niger* are two important life-threatening systemic human pathogens. More effective antifungal agents are urgently needed for the

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treatment of patients with these opportunistic infections. As an initial step toward exploring the possibility of discovering topoisomerase I-targeting antifungals, we attempted to isolate nuclear topoisomerase I from *Aspergillus nidulans*. Using the relaxation assay, we have partially purified and characterized the enzyme. Strikingly, *Aspergillus* topoisomerase I is completely resistant to some of the most potent human topoisomerase I poisons such as nitidine and coralyne, while it remains sensitive to other human DNA topoisomerase I poisons such as camptothecin and bi- and terbenzimidazoles.

MATERIALS AND METHODS

Strains, Chemicals, and Drugs. A. nidulans strain R21 (*pabaA1, yA2*) was used throughout this work. The bibenzimidazole Hoeschst dye 33342 (Ho33342), camptothecin, and berenil were purchased from Sigma Chemical Co. Monobenzimidazoles (QS/II/9, /50, /51, and /59A), terbenzimidazoles (QS/I/80 and QS/II/48), protoberberines (coralyne, DM/II/33), and nitidine were synthesized as described (see Figure 8 for structures) (Sun *et al.*, 1994, 1995; Kim *et al.*, 1996; Makhey *et al.*, 1995, 1996). All the drugs were dissolved in dimethyl sulfoxide (Sigma Chemical Co.) at a concentration of either 1, 5, or 10 mg/mL and kept frozen in aliquots at −20 °C.

Partial Purification of Topoisomerase I from A. nidulans. Two liters of YG medium (0.5% yeast extract and 2% glucose) was inoculated with approximately 5×10^8 conidia/mL. After 16 h of growth at 37 °C, the mycelia were collected, washed with buffer I [50 mM Tris-HCl (pH 7.7), 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1 mM phenylmethanesulfonyl fluoride, and 1 mM 2-mercaptoethanol], and quickly chilled in liquid nitrogen. The frozen mycelia (approximately 20 g) were ground to powder and resuspended in 300 mL of buffer I. The lysate was centrifuged at 10K rpm in a Sorval HB3 rotor for 15 min to remove cell debris. The supernatant was made 6% polyethylene glycol (v/v) and 1 M NaCl. After 1 h on ice with gentle stirring, the solution was centrifuged at 14K rpm in a Sorvall rotor for 30 min to remove nucleic acids. Subsequent steps in purification were the same as described previously for purification of recombinant human DNA topoisomerase I (Gatto *et al.*, 1996). Briefly, the supernatant was chromatographed directly onto a hydroxyapatite Bio-gel HTP (BioRad Laboratories, Richmond, CA) column. Fractions containing relaxation activity were pooled, diluted, and then loaded onto a BioRex70 (BioRad Laboratories) column. The column was developed with a linear gradient from 0.2 to 1 M KCl. The peak fractions were pooled and dialyzed overnight at 4 °C against 30 mM potassium phosphate, 50% glycerol (v/v), 0.5 mM EDTA, and 1 mM DTT. Recombinant human topoisomerase I was purified from *Escherichia coli* BL21 (DE3) harboring PET1B as described previously (Gatto *et al.*, 1996).

Covalent Transfer of ^{32}P Radioactivity from DNA to Topoisomerase I. This phosphate-transfer method was a modification of the procedure described previously (Rowe *et al.*, 1984). Briefly, a 100 μl reaction mixture containing 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 30 μg/mL bovine serum albumin, drug (camptothecin or Hoechst 33342) at an indicated concentration, 50 ng of YEpG DNA labeled with [^{32}P]dATP by the random primer method (Random Primed Labeling Kit, Boehringer Mannheim), and 300 units of human or *Aspergillus* topoisomerase I was incubated at 37 °C for 10 min. The reactions were terminated by adding NaOH to 0.18 M and EDTA to 2.5 mM. After neutralization of the reaction with a precalibrated amount of Tris-HCl, 9 μL of 0.1 M $CaCl_2$ and 7.5 μL of 20% SDS were added, and the volume was adjusted to 300 μL with H_2O. Five units of *Bal*31 nuclease (New England BioLabs) was added, and the sample was digested for 1 h at 25 °C. The reaction was terminated by extraction with 1 volume of phenol. The phenol phase was saved and back-extracted once with an equal volume of 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. The protein−oligonucleotide complexes were then precipitated from the phenol phase by adding 10 volumes of ice-cold acetone and placing on ice for 10 min. The pellet was dissolved in SDS sample buffer and analyzed by SDS−PAGE. Gel drying and autoradiography were done as described (Hsiang *et al.*, 1985).

Topoisomerase I Relaxation Assay. The relaxation assay was done as described previously (Liu & Miller, 1981). Briefly, each reaction mixture (20 μL) contained a mixture of relaxed and supercoiled YEpG DNA (150 ng each) and 1 μL of *Aspergillus* or human topoisomerase I diluted to various extents. Following an incubation at 23 or 37 °C for 15 min, the reactions were terminated by the addition of 5 μL of a prewarmed stop solution (5% sarkosyl, 25% sucrose, 50 mM EDTA, and 0.05 mg/mL bromphenol blue). DNA samples were then analyzed by using a 1% agarose gel in TPE (90 mM Tris-phosphate and 2 mM EDTA at pH 8.0) electrophoresis solution.

Topoisomerase I Cleavage Assay. DNA topoisomerase I cleavage assays were performed as described previously (Hsiang *et al.*, 1985). YEpG DNA was linearized with *Bam*HI and then 3′-end-labeled with Klenow polymerase and [α-^{32}P]dCTP. Following phenol extraction and ethanol precipitation, the labeled DNA was resuspended in 10 mM Tris (pH 8.0) and 1 mM EDTA. The DNA cleavage assay was done in a reaction mixture (20 μL) containing 40 mM Tris-HCl (pH 7.8), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg/mL bovine serum albumin, 20 ng of labeled YEpG DNA, and 1 μL of *Aspergillus* or human topoisomerase I diluted to various extents. Following incubation at 23 °C for 15 min, the reactions were terminated by the addition of SDS (final concentration of 1%) and proteinase K (final concentration of 200 μg/mL). Proteinase K treatment continued at 37 °C for an additional 1 h. The terminated reactions were either alkali-denatured and then loaded (alkaline loading) (Hsiang *et al.*, 1985) or loaded directly in neutral loading buffer (neutral loading) onto a 1% agarose gel in neutral TPE electrophoresis solution. Gel drying and autoradiography were performed as described (Hsiang *et al.*, 1985).

Yeast Cytotoxicity Assay. The topoisomerase I-specific in vivo cytotoxicity assay was adapted from Knab *et al.* (1993). In this system, various topoisomerase I genes or cDNAs cloned into the single-copy yeast plasmid vector (YCpGAL1; Knab *et al.*, 1993) are expressed under the control of the GAL1 promoter in the JN2-134 strain of *S. cerevisiae* (*MAT, rad52::LEU2, trp1, ade2-1, his7, ura3-52, ise1, top1-1, leu2*; Bjornsti *et al.*, 1989). The topoisomerase I gene or cDNA constructs in the vector are, respectively, the wild-type yeast topoisomerase I gene (YCpGAL-ScTOP1; Kim & Wang, 1989), a nonfunctional topoisomerase I gene where the active site tyrosine-727 is

mutated to phenylalanine (YCpGAL1-Sctop1Y727; Knab *et al.*, 1993), and the wild-type human topoisomerase I cDNA (YCp-GAL-hTOP1; Bjornsti *et al.*, 1989). To qualitatively test the cytotoxicity and the topoisomerase I specificity of the drugs, yeast cells containing the specified plasmid were grown in dropout medium supplemented with uracil, 2% galactose, and the drug being tested. It has been established that yeast can survive when topoisomerase I function is obliterated [Nitiss & Wang, 1988; for a review, see Nitiss (1994)] and that topo I poisons only kill cells having a functional topoisomerase I (Bjornsti *et al.*, 1989). Thus, comparison of the relative extent of growth of each of the test strains in the presence of various drugs with that in control plates (no drug) shows (a) whether the drug has any cytotoxic effects on yeast, (b) whether the cytotoxicity is topoisomerase I-specific, and (c) whether there is any differential specificity of the drugs for yeast compared with human topoisomerase I.

RESULTS

Partial Characterization of Topoisomerase I from A. nidulans. The plasmid relaxation activity was used to monitor *Aspergillus* topoisomerase I during purification. The relaxation activity in *Aspergillus* cell extract was purified through a procedure designed for purification of recombinant human DNA topoisomerase I from *E. coli* (Gatto *et al.*, 1996). Several pieces of evidence suggest that the partially purified *Aspergillus* enzyme is the major nuclear topoisomerase I identified and characterized in other eukaryotic organisms including yeast [reviewed in Wang (1996)]. First, the purified enzyme is highly active and represents the major DNA relaxation activity in *Aspergillus* cell extract. From 2 L of culture, we obtained 30 000 units of topoisomerase I relaxation activity (Figure 1A). Like human topoisomerase I, the *Aspergillus* enzyme relaxes plasmid DNA to completion (Figure 1A) and requires neither Mg(II) nor an energy cofactor (data not shown). Second, the purified *Aspergillus* enzyme relaxed both negatively (Figure 1A) and positively supercoiled DNA, a property shared by all eukaryotic nuclear DNA topoisomerase I (Figure 1B) (Wang, 1985). Third, the *Aspergillus* enzyme is sensitive to inhibition by camptothecin and Hoechst 33342 (Ho33342) which are known to inhibit (poison) human nuclear topoisomerase I (see Figures 2 and 3). The sensitivity of the *Aspergillus* enzyme to camptothecin and Hoechst 33342 was initially indicated by a phosphate-transfer experiment which was designed to determine the approximate molecular mass (M_r) of the enzyme (Figure 2). In this experiment, ^{32}P-labeled DNA was reacted with *Aspergillus* topoisomerase I to form covalent protein−DNA complexes. The covalent complex of topoisomerase I−DNA was digested with *Bal*31 to reduce the size of the labeled oligonucleotide which is covalently linked to topoisomerase I. As shown in Figure 2, using this phosphate-transfer method, *Aspergillus* topoisomerase I was identified as a 105 kDa protein which is slightly larger than recombinant human topoisomerase I (100 kDa). The lower band at the approximately 75 kDa position is known to be a proteolytic degradation product of 100 kDa human topoisomerase I. The effect of the residual oligonucleotide on the mobility of topoisomerase I is apparently negligible. Interestingly, both camptothecin (100 μM) and Ho33342 (1 μM) stimulated the phosphate transfer as shown by the enhanced labeling of 105 kDa *Aspergillus* topoisomerase I (Figure 2). At higher

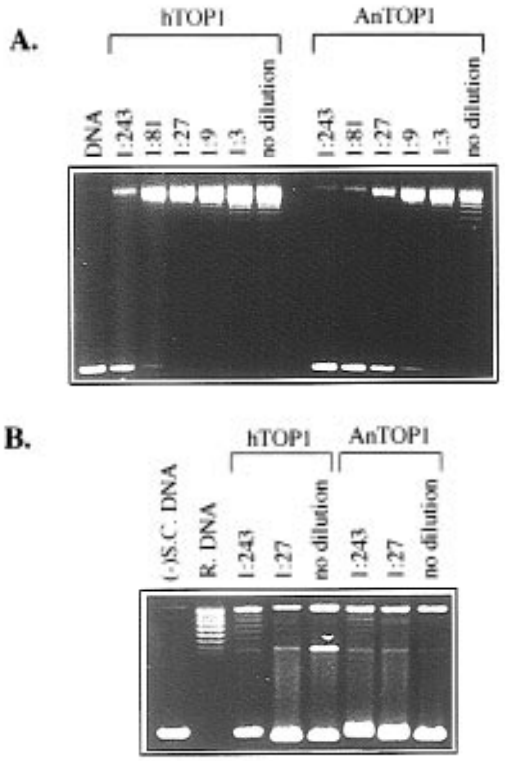

FIGURE 1: Relaxation activity of partially purified *Aspergillus* topoisomerase I. The plasmid DNA relaxation assay was performed as described in Materials and Methods. (A) Recombinant human topoisomerase I and *Aspergillus* topoisomerase I were serially diluted for comparison of their relaxation activities. One unit of enzyme activity was defined as the amount to causing 50% relaxation of the supercoiled plasmid DNA under our assay conditions. Undiluted *Aspergillus* and human topoisomerase I were estimated to be 27 and 240 units/mL, respectively. (B) Relaxation of positively supercoiled DNA by *Aspergillus* topoisomerase I. Relaxed YEpG DNA was prepared by relaxation with human DNA topoisomerase I followed by extraction with phenol and precipitation with ethanol. The relaxed YEpG DNA (0.3 μg/lane) was treated with *Aspergillus* (AnTOP1) or human (hTOP1) topoisomerase I in a standard relaxation reaction mixture containing 0.1 μg of ethidium bromide. Following incubation at 37 °C for 15 min, the reaction mixtures were extracted with phenol and precipitated with ethanol. Electrophoresis was done as described (Hsiang *et al.*, 1985).

concentrations of Ho33342, the phosphate transfer was progressively inhibited. This effect of camptothecin and Ho33342 is discussed below.

Camptothecin and Ho33342 Are Potent Inhibitors of Aspergillus Topoisomerase I. The phosphate-transfer experiment has suggested that both camptothecin and Ho33342 may inhibit *Aspergillus* topoisomerase I by a poisoning mechanism. In order to test this possibility, *Aspergillus* topoisomerase I was used in a DNA cleavage reaction in the presence of various drugs. As shown in Figure 3, both camptothecin (CPT) and Ho33342 (HOE) are potent inhibitors of *Aspergillus* topoisomerase I. Extensive DNA cleavage was observed at concentrations as low as 2.8 and 0.17 μM for camptothecin and Ho33342, respectively. Interestingly, nitidine and coralyne, which are known to be highly potent inhibitors of human DNA topoisomerase I, did not inhibit *Aspergillus* topoisomerase I to any significant extent (Figure 3). DM/II/33, another highly potent inhibitor of human DNA topoisomerase I, was only weakly inhibitory toward *Aspergillus* topoisomerase I. Berenil, which is inactive against human topoisomerase I, was also inactive against *Aspergillus* topoisomerase I. These results indicate

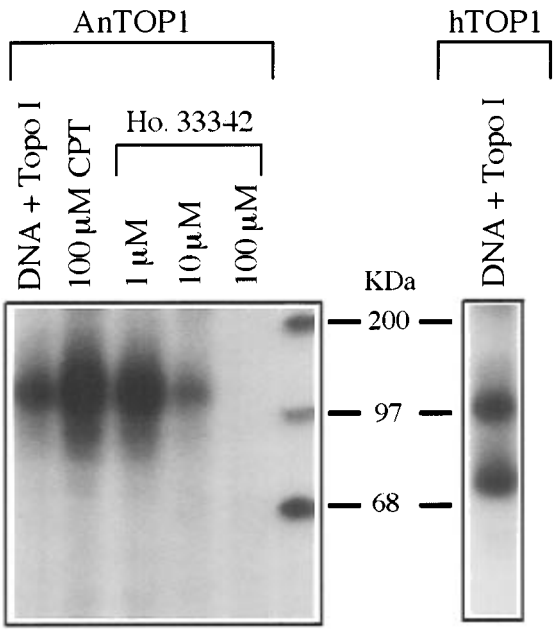

FIGURE 2: Estimation of the molecular mass of *Aspergillus* topoisomerase I by a phosphate-transfer method. Covalent topoisomerase I−DNA complexes were processed by *Bal*31 nuclease digestion and electrophoresed in a SDS−polyacrylamide gel as described in Materials and Methods. The lane on the right labeled hTOP1 showed two labeled bands, representing 100 kDa human topoisomerase I and its proteolyzed fragment of 75 kDa. The lanes in the left panel showed the labeled protein bands representing *Aspergillus* topoisomerase I (AnTOP1). The reaction mixtures containing various inhibitors are labeled at the top of each lane.

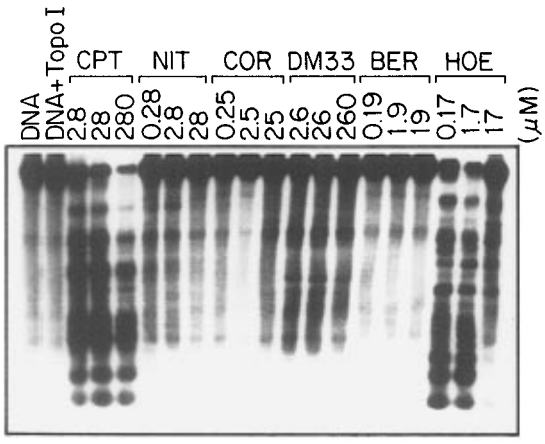

FIGURE 3: Aspergillus topoisomerase I is selectively sensitive to some human topoisomerase I poisons. The DNA cleavage assay was performed as described in Materials and Methods. Sixty units of *Aspergillus* topoisomerase I was used in each reaction. Reactions were terminated and the mixtures denatured by an alkali−SDS solution and analyzed with a 1% agarose gel in neutral TPE buffer (Hsiang *et al.*, 1985). The DNA fragments in the gel are single-stranded due to the alkaline loading solution (NIT, nitidine; COR, coralyne; DM33, DM/II/33; BER, berenil; and HOE, Hoechst 33342).

that human and *Aspergillus* topoisomerase I are quite different in terms of their sensitivity toward various enzyme inhibitors. It is also interesting to note that, at the highest concentration of Ho33342 (17 μM), topoisomerase I-mediated DNA cleavage was dramatically inhibited. This cleavage-inhibitory effect at higher concentrations of inhibitors has been described previously for a number of intercalators and DNA minor groove binding ligands (Chen *et al.*, 1993) and attributed to inhibition of enzyme binding to the DNA

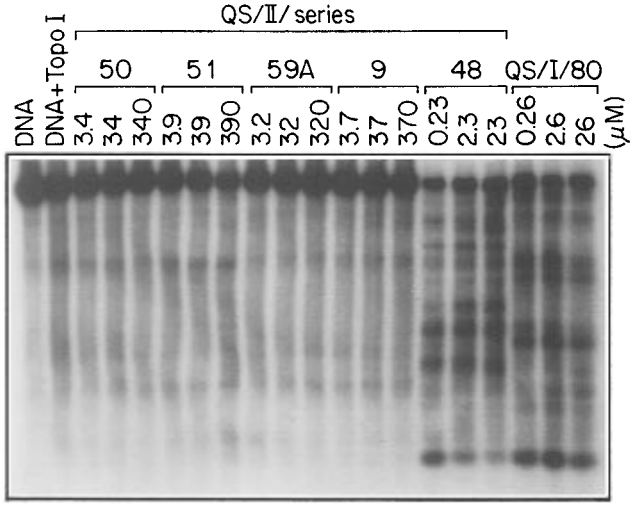

FIGURE 4: Selective sensitivity of *Aspergillus* topoisomerase I to ter- but not monobenzimidazoles. Various mono- (QSII/50, QS/II/51, QS/II/59A, and QS/II/9) and terbenzimidazoles (QS/II/48 and QS/I/80) were used in this cleavage assay using *Aspergillus* topoisomerase I (60 units/reaction). The cleavage assay was performed as described in Materials and Methods.

template (Tewey *et al.*, 1985; Chen *et al.*, 1993). The inhibitory effect of Ho33342 on phosphate transfer to *Aspergillus* topoisomerase I (shown in Figure 2) can therefore be similarly explained.

Selective Sensitivity of Aspergillus Topoisomerase I to Bi- and Terbenzimidazoles. Previous studies have identified a number of mono-, bi-, and terbenzimidazoles as effective inhibitors (poisons) of mammalian DNA topoisomerase I (Chen *et al.*, 1993; Sun *et al.*, 1994, 1995; Kim *et al.*, 1996a,b). To test whether *Aspergillus* topoisomerase I is also sensitive to the inhibitory effect of these benzimidazoles, a number of compounds were screened using the cleavage assay. As shown in Figure 4, *Aspergillus* topoisomerase I was strongly inhibited (poisoned) by QS/II/48 and QS/I/80, both of which are terbenzimidazoles. None of the monobenzimidazoles, including QS/II/50, QS/II/51, QS/II/59A, and QS/II/9, exhibited any inhibitory effect on *Aspergillus* topoisomerase I. Previous studies have established that all these monobenzimidazoles except QS/II/50 are inhibitors (poisons) of mammalian DNA topoisomerase I (Kim *et al.*, 1996a). The selective sensitivity of *Aspergillus* topoisomerase I to bi- (e.g. Ho33342) and ter- (e.g. QS/II/48 and QS/I/80) but not monobenzimidazoles (e.g. QS/II/9) again indicates differences in drug sensitivity between the human and *Aspergillus* enzymes.

Differences in Cleavage Specificity between Human and Aspergillus Topoisomerase I. In addition to differences in drug sensitivity between human and *Aspergillus* topoisomerase I, additional differences in cleavage specificity have been observed between human and *Aspergillus* topoisomerase I. As shown in Figure 5, the cleavage patterns of human and *Aspergillus* enzymes are dramatically different in the presence of the bibenzimidazole Ho33342 (HOE). The larger number of cleavage sites and the larger extent of cleavage exhibited by *Aspergillus* topoisomerase I in the presence of HOE are not understood. Although less obvious, the cleavage patterns of the human and *Aspergillus* enzymes were also different in the presence of camptothecin (CPT) (Figure 5). To rule out the possibility that contaminating topoisomerase II in the *Aspergillus* topoisomerase I enzyme

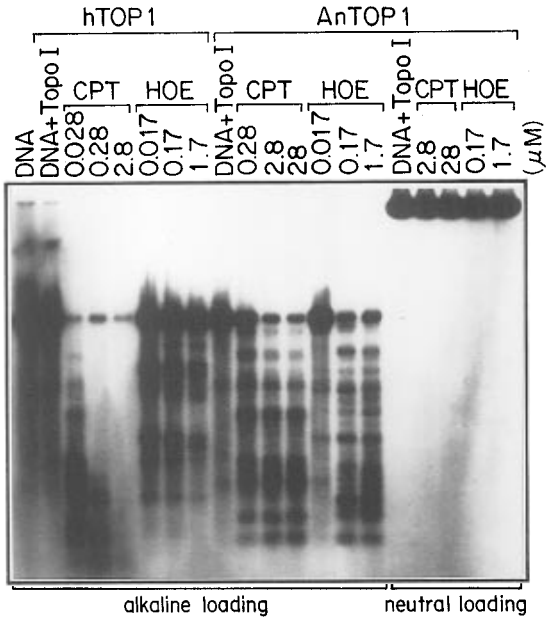

FIGURE 5: *Aspergillus* topoisomerase I exhibits a cleavage specificity different from that of human topoisomerase I in the presence of the bibenzimidazole Ho33342. Both human (labeled hTOP1, 150 units/reaction) and *Aspergillus* (labeled AnTOP1, 60 units/reaction) were used for the cleavage assay described in Materials and Methods. Different concentrations of camptothecin (CPT) and Ho33342 (HOE) were used as indicated at the top of each lane.

preparation may contribute to the cleavage pattern, part of the samples was also analyzed for possible double-stranded breaks. As shown in Figure 5, no double-stranded DNA breaks were observed when DNA samples were analyzed by neutral rather than alkaline loading. It is also evident from this experiment that *Aspergillus* topoisomerase I is less sensitive to CPT than the human enzyme.

The differences in cleavage specificity between human and *Aspergillus* enzymes were also quite evident when terbenzimidazoles (QS/II/48 and QS/I/80) were used (Figure 6). In addition, the *Aspergillus* enzyme appeared to be substantially more sensitive to QS/II/48 than the human enzyme.

Yeast and Aspergillus Topoisomerase I Enzymes Exhibit Similar Drug Sensitivity/Resistance. Yeast top1 deletion strains expressing human or yeast DNA topoisomerase I under identical conditions have been used to evaluate differential drug sensitivity of the human and yeast enzymes (Nitiss & Wang, 1988; Gatto *et al.*, 1996). As previously shown (Nitiss, 1994), although yeast cells expressing yeast topoisomerase I are camptothecin-sensitive, they are at least 10 times more resistant to camptothecin than yeast cells expressing human topoisomerase I (Figure 7, left three panels). As shown in Figure 7 (right three panels), nitidine, DM/II/33, and QS/II/9 are highly cytotoxic against yeast cells expressing human topoisomerase I but not cytotoxic against yeast cells expressing either functional or nonfunctional yeast topoisomerase I (at least a 600-fold difference in sensitivity was observed). These results support the notion that yeast and *Aspergillus* topoisomerase I are resistant to the same drugs (i.e. nitidine, the protoberberine DM-II-33, and the monobenzimidazole QS-II-9) that poison human topoisomerase I.

DISCUSSION

There is an increasing need for novel antifungal agents to treat the growing population of patients at risk for systemic

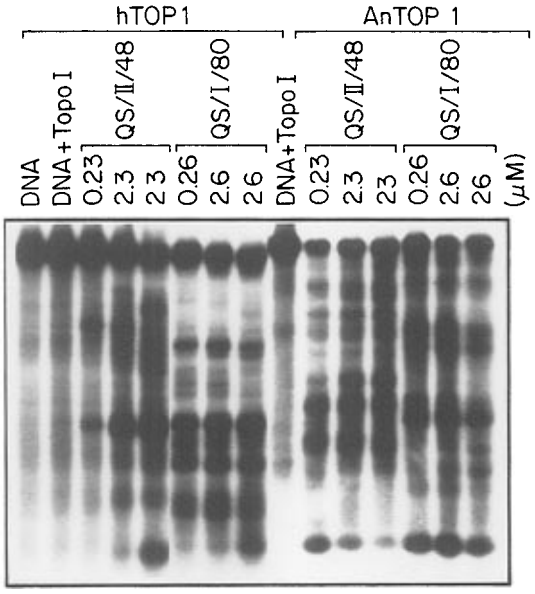

FIGURE 6: *Aspergillus* topoisomerase I exhibits a cleavage specificity different from that of human topoisomerase I in the presence of terbenzimidazoles. Both human (150 units/reaction) and *Aspergillus* (60 units/reaction) enzymes were used in the cleavage reaction described in Materials and Methods. The concentrations of the terbenzimidazoles, QS/II/48 and QS/I/80, are indicated at the top of each lane.

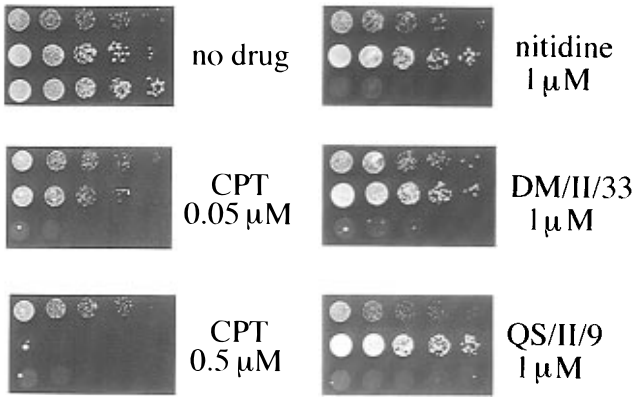

FIGURE 7: Both *Aspergillus* and *S. cerevisiae* topoisomerase I enzymes are resistant to the same human topoisomerase I poisons. The yeast strain JN2-134 lacking the chromosomal copy of the topoisomerase I gene was transfected with the following (in each panel): top row, a mutated (active site tyrosine mutant) yeast topoisomerase I gene; middle row, the wild-type yeast topoisomerase I gene; and bottom row, the wild-type human topoisomerase I gene. Yeast cells were serially (5-fold) diluted and grown on minimal plates containing 2% galactose and uracil as a selection marker. The plates also contained various drugs as indicated to the right of each panel. The concentrations of the drugs in plates are as follows: no drug, 0.05 and 0.5 μM camptothecin, 1 μM nitidine, 1 μM DM/II/33, and 10 μM QS/II/9.

fungal infections. The choices for antifungal therapies are limited, and fungal strains resistant to the current therapies have become an increasing problem in hospitals (Fostel *et al.*, 1992). There is a need for new antifungal therapies, and topoisomerases have been suggested as important therapeutic targets for new antifungal agents (Fostel *et al.*, 1992; Fostel & Montgomery, 1995; Shen & Fostel, 1994; Shen *et al.*, 1992). To establish topoisomerase I as a useful target for antifungal agents, the antifungal agents must specifically inhibit the fungal enzyme with a minimal effect on the host (human) enzyme. As an initial step toward discovering a topoisomerase I-targeting antifungal, we puri-

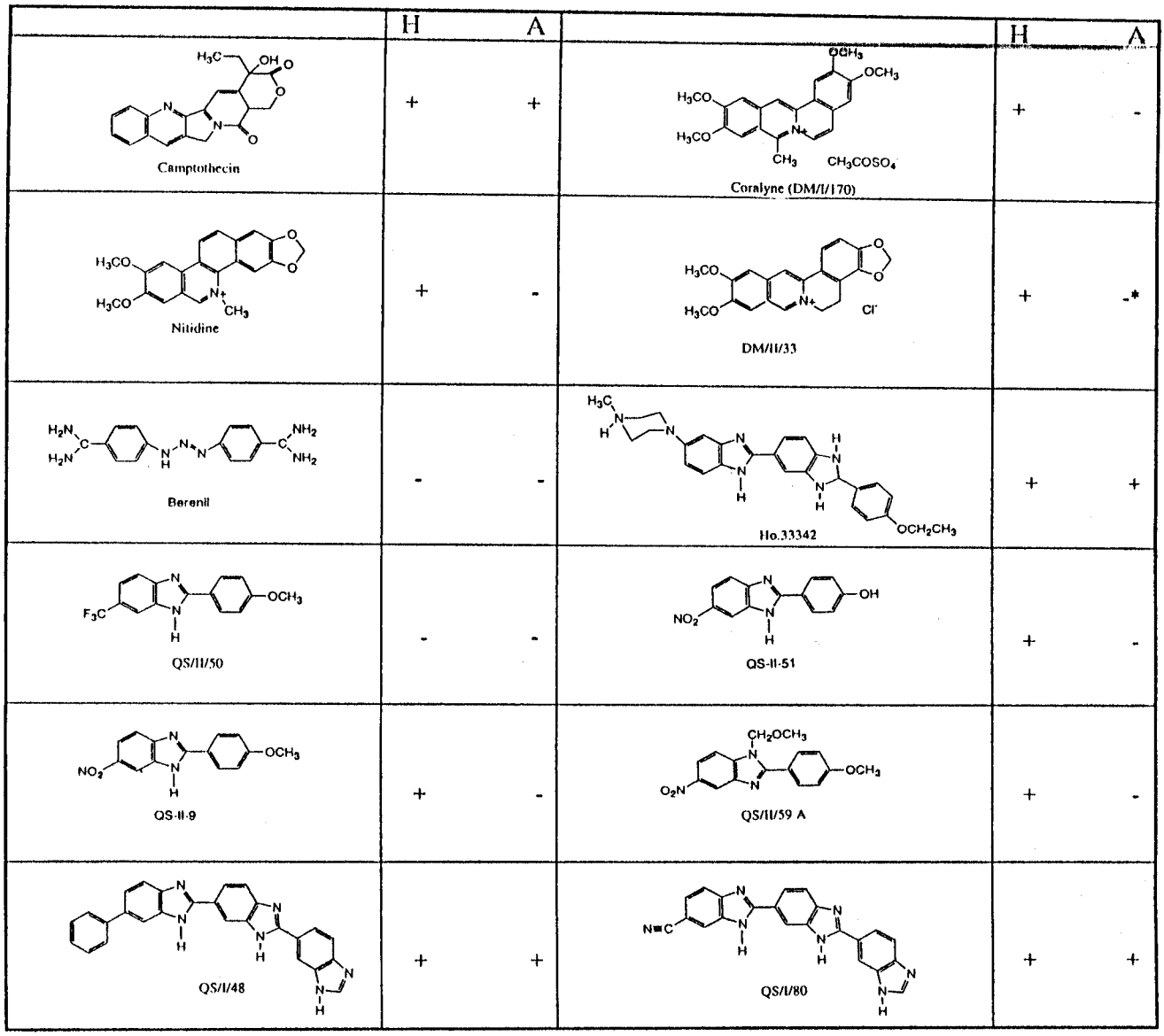

FIGURE 8: Summary of the activity of various drugs against human and *Aspergillus* topoisomerase I. The poisoning activities of various drugs against human (H column) and *Aspergillus* (A column) are qualitatively indicated by a + (active) or − (inactive). DM/II/33 is only very weakly active against *Aspergillus* topoisomerase I and is indicated with an *.

fied *A. nidulans* topoisomerase I and compared its cleavage activity and pattern with those of the human counterpart. On the basis of the phosphate-transfer experiment, the M_r of *A. nidulans* topoisomerase I was estimated to be 105 kDa. Using the same method, recombinant human topoisomerase I exhibited a M_r of 100 kDa, slightly smaller than the *Aspergillus* enzyme, but consistent with the reported reduced molecular mass of HeLa topoisomerase I (Liu & Miller, 1981). The topoisomerase I enzymes isolated from *S. cerevisiae* and *C. albicans* have been shown to have reduced molecular masses of 95 and 102 kDa, respectively, on the basis of SDS−PAGE. The differences in molecular mass among these different eukaryotic type I topoisomerases can be understood once the sequence of the *Aspergillus* topoisomerase I is determined. We are currently cloning the *Aspergillus* topoisomerase I gene by the PCR method and have obtained a 1.4 kb fragment with 78% amino acid similarity (66% identity) to topoisomerase I from *S. cerevisiae* (M. Sanders, unpublished results).

Aspergillus topoisomerase I, like human topoisomerase I, is sensitive to the poisoning activity of camptothecin, the bibenzimidazole Ho33342, and terbenzimidazoles (QS/II/48 and QS/I/80) (Figure 8). Although camptothecin appears to be less active against the *Aspergillus* enzyme than the human enzyme, the terbenzimidazole QS/II/48 appears to be more active against the *Aspergillus* enzyme than the human enzyme (Figure 8). The effectiveness of the terbenzimidazoles against *Aspergillus* topoisomerase I is not restricted to QS/II/48 and QS/I/80; a number of other terbenzimidazoles are also highly effective against the fungal enzyme (unpublished results). The general higher sensitivity of *Aspergillus* topoisomerase I to terbenzimidazoles is not understood. However, as judged from the higher extent of cleavage and looser sequence specificity of cleavage, one may argue that *Aspergillus* topoisomerase I may be less sensitive to the inhibitory effect of these DNA binding ligands. This could be explained if *Aspergillus* topoisomerase I binds DNA with higher affinity than the human enzyme and therefore is less susceptible to the inhibitory effect of these DNA binding ligands. We are currently testing this hypothesis.

One striking finding from the current study is that the *Aspergillus* enzyme is completely resistant to some of the most potent human topoisomerase I poisons such as nitidine and coralyne (Figure 8; Wang *et al.*, 1993, 1996; Makhey *et al.*, 1996; Gatto *et al.*, 1996) and to the less potent human topoisomerase I-poisoning monobenzimidazoles (Figure 8; Kim *et al*., 1996a). Studies using yeast expressing human or yeast topoisomerase I have also suggested a similar resistance of the yeast topoisomerase I to these compounds. It appears that the fungal enzymes are quite different in their drug sensitivity from their human counterpart. This striking difference in drug sensitivity may assure the possibility of discovering topoisomerase I-specific antifungals in the future.

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