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Genetics

Bacteriophage T4 DNA topoisomerase is the target of antitumor agent 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) in T4-infected *Escherichia coli*

(acridine/DNA cleavage/DNA relaxation/drug resistance)

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ABSTRACT **The mammalian type II DNA topoisomerase has been proposed to be the intracellular target of a variety of antitumor agents, including *m*-AMSA [4′-(9-acridinylamino)-methanesulfon-*m*-anisidide]. Because the bacteriophage T4-encoded topoisomerase resembles the mammalian enzyme, we are using T4 as a simple model system to investigate the mechanism of action of *m*-AMSA. A mutation that renders T4 growth *m*-AMSA-resistant is closely linked to an amber mutation in T4 gene *39*, which encodes one of the topoisomerase subunits. In addition, the gene *39* subunit from the *m*-AMSA-resistant mutant phage has an altered net charge, strongly indicating that the drug-resistance mutation is within gene *39*. Topoisomerase purified from mutant phage-infected *Escherichia coli* exhibits drug-insensitive DNA relaxation and DNA cleavage activities. Because a single mutation results in both drug-resistant phage growth and a drug-insensitive viral topoisomerase, we conclude that the T4-encoded type II DNA topoisomerase is the physiological target of *m*-AMSA in phage-infected *E. coli*.**

The mammalian type II DNA topoisomerase has been proposed to be the intracellular target of several classes of antitumor agents, including acridine derivatives, anthracyclines, and certain epipodophyllotoxins (for review, see ref. 1). These agents block topoisomerase activity *in vitro* and cause the accumulation of covalent complexes consisting of topoisomerase attached to each 5′ terminus of double-strand DNA breaks (2, 3). Because the type II topoisomerase catalyzes passage of one duplex DNA segment through a transient double-strand break in a second DNA segment, the drug-induced covalent complexes are thought to be identical to an intermediate in the normal strand-passage reaction (3). The accumulation of covalent complexes in the presence of these agents suggests that the drugs block the resealing step of the topoisomerase reaction cycle.

Although it is clear that topoisomerase is inhibited by these various antitumor agents, a definitive assignment of the specific intracellular target has not been made. Some of these agents inhibit other important cellular enzymes, such as DNA polymerase (4, 5). In addition, the quantity of protein-linked DNA breaks does not always correlate with cytotoxicity (6, 7), and protein-free DNA breaks have been detected in drug-treated cells (4, 7). Although studies using drug-resistant mammalian cell lines are consistent with the model that topoisomerase is the target of drug action (8–12), in no case has a single mutation been shown to confer both drug-resistant cell growth and drug-resistant topoisomerase activity.

To circumvent problems encountered in complex mammalian systems, we used the simple bacteriophage T4 (13) as a model system to investigate the mechanism of action of the antitumor agent 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA). Many of the properties of the T4 topoisomerase are similar to those of the mammalian, but not the bacterial, type II topoisomerase (14, 15). Most importantly, both the mammalian and phage enzymes are sensitive to *m*-AMSA *in vitro* (3, 16); *m*-AMSA treatment results in the production of topoisomerase-linked DNA breaks by both enzymes.

Similarities between nucleic acid metabolism in mammalian cells and phage T4-infected bacterial cells are not limited to the DNA topoisomerase. The T4 DNA polymerase demonstrates greater homology with mammalian DNA polymerase α than with *Escherichia coli* DNA polymerases (17, 18). Further, introns processed at the level of mRNA have been found in several T4 genes (19–21). Given these and other similarities, phage T4 constitutes a uniquely informative model system.

In this report, we demonstrate that T4 DNA topoisomerase is the intracellular target of *m*-AMSA in T4-infected *E. coli* and describe the properties of an *m*-AMSA-resistant (*m*-AMSA^R) topoisomerase. These findings should facilitate determination of the detailed mode of action of *m*-AMSA.

MATERIALS AND METHODS

Materials. The wild-type and *m*-AMSA^R mutant topoisomerases were purified essentially as described (14), using a gene *33 55* amber (*am*) genetic background (T4 33^{am} 55^{am}) to maximize yield. Restriction enzymes were purchased from New England Biolabs. *m*-AMSA (NCS-249992) was obtained from the National Cancer Institute.

Strains. *E. coli* CR63 (*supD*), *E. coli* B_E (nonsuppressing), T4$^+$D (wild type), T4 *amN116* (gene *39*), T4 *amH17* (gene *52*), and T4 *amN134 amBL292* (genes *33* and *55*) were originally from the collection of B. M. Alberts (University of California, San Francisco).

DNA Relaxation Assays. Reaction mixtures (20 μl) contained 40 mM Tris·HCl (pH 7.8), 60 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 0.5 mM Na_2EDTA, nuclease-free bovine serum albumin (30 μg/ml), negatively supercoiled pBR322 DNA (0.3 μg), and the indicated amount of topoisomerase. After 30 min at 30°C, Na_2EDTA (final concentration, 45 mM) was added to terminate each reaction; Na_2EDTA minimizes topoisomerase-cleaved DNA products (22). Gel-loading buffer [5 μl containing 5% (wt/vol)

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Abbreviations: *m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide; *m*-AMSA^R, *m*-AMSA-resistant (ce); *am*, amber; gp, gene product.

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$NaDodSO_4$, 20% (wt/vol) Ficoll, 0.1% bromophenol blue, and 0.1% xylene cyanol] was then added to each reaction mixture, and the products were incubated for 1 hr at 37°C in the presence of proteinase K (Boehringer Mannheim; 100 μg/ml). Half of each sample was subjected to electrophoresis through a 0.8% agarose gel in TBE running buffer (89 mM Tris base/89 mM boric acid/2.5 mM Na_2EDTA) for ≈16 hr at 2 V/cm. The other half was resolved by electrophoresis through a 1% agarose gel containing chloroquine diphosphate (23) (7.5 μg/ml; Sigma) for ≈24 hr at 2 V/cm. The TBE running buffer contained the same concentration of chloroquine diphosphate and was recirculated. DNA was visualized by UV illumination after ethidium bromide staining.

DNA Cleavage Assays. *Eco*RI-linearized pBR322 DNA was radiolabeled by filling-in the recessed 3′ termini by using the Klenow fragment of *E. coli* DNA polymerase I (United States Biochemical) in the presence of [α-^{32}P]dATP (Amersham). Subsequent digestion with *Hin*dIII yielded a 4336-base-pair fragment uniquely labeled at the *Eco*RI-cleaved terminus. A portion of this radioactive DNA, combined with 0.3 μg of identically digested unlabeled pBR322 DNA, was incubated with the indicated amounts of topoisomerase in the presence or absence of *m*-AMSA (5 μg/ml). Assays were otherwise identical to DNA relaxation assays. After 30 min at 30°C, reactions were terminated by the addition of the $NaDodSO_4$-containing gel loading buffer, and the products were treated with proteinase K for 1 hr at 37°C to eliminate covalently attached topoisomerase.

Nonequilibrium pH-Gradient Gel Electrophoresis. Samples of the wild-type and mutant topoisomerases were electrophoresed through a nonequilibrium pH-gradient gel containing 8 M urea, 5.5% (wt/vol) acrylamide, and 2% (wt/vol) ampholytes [Bio-Rad; the ratio of ampholytes (pH 5–7/pH 3.5–10) was 3:2]. Protein samples were electrophoresed from anode to cathode for a total of 1200 V·hr. The gel was soaked overnight in 20% (wt/vol) trichloroacetic acid and then for 2 hr in 10% (vol/vol) acetic acid/45% (vol/vol) methanol to remove ampholytes prior to staining with Coomassie brilliant blue R-250 (Bio-Rad).

RESULTS

T4 Mutants Resistant to *m*-AMSA. Wild-type T4 failed to form plaques in the presence of *m*-AMSA (at concentrations as low as 50 μg/ml), while growth of the *E. coli* host apparently was unaffected by drug concentrations up to at least 150 μg/ml. Therefore, *m*-AMSA^R phage mutants were selected simply by plating hydroxylamine-mutagenized phage stocks on lawns of *E. coli* in the presence of drug.

Several genetic crosses were performed using an *m*-AMSA^R mutant phage that demonstrated a high level of drug resistance. To determine if resistance resulted from a single or multiple mutations, two successive backcrosses were conducted with a 10-fold excess of drug-sensitive phage. In each cross, roughly 10% of the progeny exhibited a high level of drug resistance (unpublished data). This is the result expected if the *m*-AMSA^R phenotype is due to a single point mutation (or very closely linked multiple mutations). Each of the two successive backcrosses should have replaced roughly 90% of the mutagenized phage genome with unmutagenized DNA.

We suspected that the phage topoisomerase is the *m*-AMSA target and, therefore, tested the *m*-AMSA^R mutation for linkage to known topoisomerase mutations. T4 topoisomerase is composed of three subunits encoded by genes *39*, *52*, and *60* (24). Because gene *60* is closely linked to gene *39*, linkage studies were conducted only with *39* and *52* amber (chain-termination) mutant phage. Host strain CR63 (*supD*) was coinfected with equal numbers of the *m*-AMSA^R and either 39^{am} or 52^{am} mutant phage, and am^+ progeny were tested for drug resistance.

A large majority (47/50 or 94%) of the am^+ progeny from the *m*-AMSA^R × 39^{am} cross were drug-resistant, indicating that the *m*-AMSA^R mutation is very closely linked to the gene 39^{am} mutation. In contrast, the *m*-AMSA^R × 52^{am} cross yielded approximately one-half (24/50) *m*-AMSA^R phage among the am^+ progeny, as would be expected if the *m*-AMSA^R and 52^{am} mutations are unlinked. The am^+ progeny of control crosses (T4$^+$D and either T4 39^{am} or T4 52^{am}) were all *m*-AMSA sensitive. These results demonstrate that the *m*-AMSA^R mutation is either within or very near the topoisomerase structural gene *39*.

***m*-AMSA^R Topoisomerase Activity.** To determine if the *m*-AMSA^R mutation results in a drug-resistant topoisomerase, we purified T4 topoisomerase from *E. coli* infected with either T4 $33^{am}55^{am}$ or T4 $33^{am}55^{am}$ *m*-AMSA^R (a progeny of the second backcross; see above). As judged by $NaDodSO_4$/PAGE, topoisomerase encoded by the *m*-AMSA^R phage is indistinguishable from wild-type enzyme (Fig. 1). Both preparations contain approximately equimolar amounts of the three known topoisomerase subunits (14), and both are essentially homogeneous.

DNA relaxation catalyzed by either enzyme preparation was found to be ATP-dependent (unpublished data), as expected from studies of the wild-type enzyme (26, 27). The specific activity of the wild-type topoisomerase (1.5×10^6 units/mg; see ref. 14 for unit definition) is about 8-fold higher than that of the enzyme purified from the *m*-AMSA^R mutant.

We next tested the *m*-AMSA-sensitivity of DNA relaxation catalyzed by the two topoisomerases. A dilution of each enzyme was chosen to yield equivalent levels of DNA relaxation in the absence of *m*-AMSA; extensive but incomplete relaxation was desired to maximize detection of enzyme inhibition. Reaction products were analyzed by electrophoresis through agarose and also through chloroquine-containing agarose to resolve various DNA forms (see Fig. 2).

DNA relaxation catalyzed by the wild-type enzyme (Fig. 2*A*, lane 3a) was demonstrated by the decreased intensity of form I (supercoiled) DNA relative to the untreated substrate

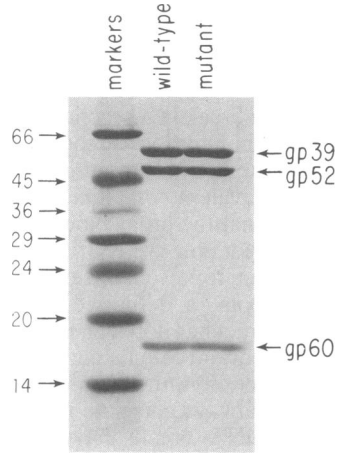

FIG. 1. Wild-type and *m*-AMSA^R mutant topoisomerases are indistinguishable by $NaDodSO_4$/PAGE. A sample of each enzyme (1.6 μg) was subjected to electrophoresis according to the procedure of Laemmli (25) using a 6% (wt/vol) polyacrylamide stacking and 12.5% (wt/vol) polyacrylamide separating gel. Resolved protein bands were visualized by staining with Coomassie brilliant blue R-250, and a photograph of the stained gel is shown. Molecular mass marker proteins (Sigma) appear in the leftmost lane, with corresponding values (in kDa) indicated alongside each band. Assignment of the three topoisomerase subunits is based on molecular mass values as estimated from this gel and published data (14). gp, Gene product.

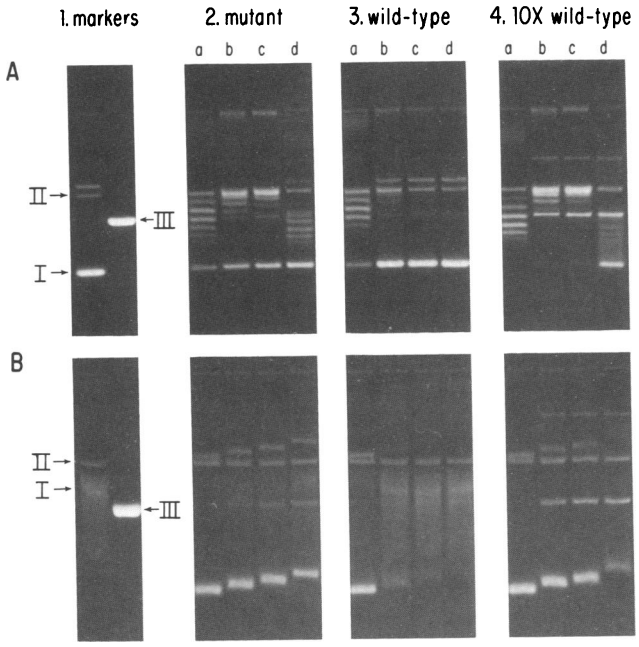

FIG. 2. DNA relaxation activity of the mutant topoisomerase is resistant to *m*-AMSA. Negatively supercoiled pBR322 DNA was treated with either wild-type or drug-resistant topoisomerase in reaction mixtures containing *m*-AMSA at concentrations of 0 (lanes a), 5 (lanes b), 10 (lanes c), or 20 (lanes d) μg/ml. Mutant topoisomerase (5.7 ng) was added to each of the reaction mixtures in lanes 2, and wild-type topoisomerase (0.64 or 6.4 ng) was added to each of the reaction mixtures in lanes 3 and 4, respectively. The reaction products were subjected to agarose gel electrophoresis in the absence (*A*) or presence (*B*) of chloroquine. Lanes 1 contain untreated substrate pBR322 DNA (left lane) and *Eco*RI-linearized pBR322 DNA (right lane). Roman numerals indicate negatively supercoiled (form I) DNA, nicked circular (form II) DNA, and *Eco*RI-linearized (form III) DNA. The intercalation of chloroquine during electrophoresis (*B*) decreased the helical winding number and consequently increased the writhing number of closed circular DNA; native supercoiled DNA thereby migrated between forms II and III, and relaxed DNA appeared as the most rapidly migrating band. In certain lanes of both gels, a small amount of *m*-AMSA-dependent, topoisomerase-cleaved linear (form III) DNA is visible.

(lane 1, left) and by the appearance of relaxed topoisomers that migrated between form II (nicked) and form I DNA. In the matching lanes of the chloroquine-containing gel, the relaxed DNA product is the most rapidly migrating band (Fig. 2*B*, lane 3a). Addition of *m*-AMSA (5, 10, or 20 μg/ml) to the wild-type topoisomerase reaction mixtures blocked DNA relaxation (Fig. 2 *A* and *B*, compare lanes 3b–3d with lane 3a).

As indicated above, a dilution of mutant phage-encoded topoisomerase was chosen that catalyzed DNA relaxation comparable to that in the wild-type enzyme assays (in Fig. 2*A* compare lanes 2a and 3a and in Fig. 2*B* compare lanes 2a and 3a). The addition of increasing concentrations of *m*-AMSA to the mutant enzyme assays only slightly inhibited, but did not eliminate, DNA relaxation (Fig. 2 *A* and *B*, compare lanes 2b–2d with lanes 2a). We conclude that topoisomerase from the *m*-AMSA^R mutant is significantly resistant to *m*-AMSA.

The relaxed DNA forms produced by the mutant topoisomerase in the presence of *m*-AMSA differed from those produced in the absence of drug. In the agarose gel without chloroquine (Fig. 2*A*), the majority of the relaxed DNA comigrated with form II (nicked) DNA at intermediate drug concentrations (lanes 2b and 2c); at the highest drug concentration, the relaxed DNA formed a distribution of bands between forms II and I (lane 2d). In the presence of chloroquine (Fig. 2*B*), the relaxed forms showed a continuously decreasing migration as the *m*-AMSA concentration increased; however, the level of nicked DNA did not change with *m*-AMSA addition (compare lanes 2a–2d). Therefore, the slowly migrating bands in Fig. 2*A*, lanes 2b and 2c, consisted of relaxed, not nicked, DNA. These alterations in the relaxed DNA products are primarily the result of interactions between *m*-AMSA and substrate DNA and do not reflect enzyme inhibition. The same patterns of relaxed DNA forms were observed when linearized substrate DNA was ligated in the presence of increasing concentrations of *m*-AMSA (unpublished data). Presumably, the intercalation of *m*-AMSA (28) decreases the number of helical twists in the DNA, so that, in the course of the topoisomerase or ligase reaction, product DNA with an average writhing number of zero accumulates. However, the subsequent loss of *m*-AMSA [during $NaDodSO_4$ treatment (29) and electrophoresis] should increase the number of helical twists and, thereby, cause a compensating decrease in the writhing number, which, in turn, affects electrophoretic mobility (see refs. 30 and 31; also see Fig. 2).

To quantitate the relative resistance of the mutant enzyme, a 10-fold greater level of the wild-type enzyme also was tested for DNA relaxation (Fig. 2 *A* and *B*, lanes 4). In this case, a substantial amount of *m*-AMSA-insensitive DNA relaxation was detected at intermediate drug concentrations (Fig. 2 *A* and *B*, lanes 4b and 4c), but relaxation was effectively inhibited at the highest *m*-AMSA concentration (lanes 4d). Therefore, the DNA relaxation activity of the drug-resistant topoisomerase is at least 10 times less sensitive to *m*-AMSA than that of the wild-type enzyme (compare Fig. 2*B*, lanes 2 and 4).

Drug-Induced DNA Cleavage Activities. As described above, *m*-AMSA induces the formation of protein-DNA complexes in which topoisomerase is covalently attached to the 5′ termini of double-strand DNA breaks. Cleavage of linear pBR322 DNA by the wild-type topoisomerase was increased at least 50-fold in response to *m*-AMSA addition

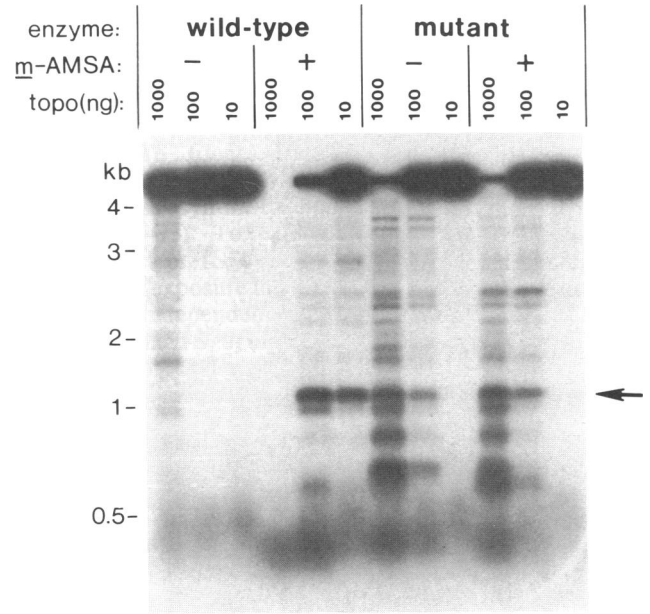

FIG. 3. DNA cleavage activity of the mutant topoisomerase is insensitive to *m*-AMSA. Uniquely end-labeled, linear pBR322 DNA was incubated with the indicated quantities of wild-type or *m*-AMSA^R mutant topoisomerase in the presence (+) or absence (–) of *m*-AMSA (5 μg/ml). Cleavage products were resolved by electrophoresis through a 0.8% agarose gel, and an autoradiogram of the gel is shown. A molecular mass scale (kb, kilobases), generated from the migration of pBR322 DNA restriction fragments, appears in the left-hand margin. No *m*-AMSA-induced DNA cleavage was detected in the absence of topoisomerase (unpublished data).

(Fig. 3). Note that in the presence of *m*-AMSA, the largest amount (1 μg) of wild-type enzyme degraded the substrate DNA predominantly to fragments <500 base pairs. In contrast, DNA cleavage by the *m*-AMSA^R mutant enzyme increased only slightly (2-fold or less) in the presence of drug (Fig. 3)—a response consistent with the drug-resistant DNA relaxation activity of the mutant enzyme. Surprisingly, in the absence of *m*-AMSA, the mutant enzyme mediated roughly 4- to 5-fold more DNA cleavage than the wild-type enzyme (Fig. 3; also see *Discussion*).

Two features of the DNA cleavage site specificities are also of interest. (*i*) *m*-AMSA changed the cleavage site specificity of the wild-type T4 enzyme. The most striking alteration was the induction of a strong cleavage site that yielded an end-labeled fragment of ≈1050 base pairs (indicated by arrow in Fig. 3). In sharp contrast, *m*-AMSA generally did not alter the cleavage site specificity of the *m*-AMSA^R mutant enzyme. (*ii*) The DNA cleavage patterns for the mutant and wild-type enzymes in the absence of *m*-AMSA did not match. Therefore, the *m*-AMSA^R mutation alters the (drug independent) DNA cleavage site specificity of the T4 topoisomerase. The cleavage pattern generated by the mutant enzyme in the absence of drug more closely matched that of the wild-type enzyme in the presence of drug; the prominent 1050-base pair fragment appeared in both cases.

Altered Gene *39* Subunit. The genetic analyses described above indicated that the *m*-AMSA^R mutation lies in either gene *39* or the neighboring gene *60*. In an effort to determine which topoisomerase subunit is altered by the *m*-AMSA^R mutation, the mutant enzyme was examined for possible charge alterations. The migration patterns of wild-type and mutant topoisomerase subunits were compared by using denaturing nonequilibrium pH-gradient gel electrophoresis (32, 33). The wild-type and mutant enzymes each formed three bands, but there was a difference in the migration of the gp39 subunit (Fig. 4). Because a mixture of mutant and wild-type enzymes yielded four distinct bands, gel migration artifacts cannot be responsible for the observed difference. This analysis strongly suggests that the *m*-AMSA^R mutation results in a charge alteration in the gene *39* subunit of the T4 topoisomerase.

DISCUSSION

We have analyzed the mechanism of action of antitumor agent *m*-AMSA in a simple bacteriophage T4 model system. Genetic and biochemical analyses demonstrated that resistance to *m*-AMSA can be conferred by a mutation in phage gene *39*, which encodes one subunit of the T4 topoisomerase. Because a single mutation results in both drug-resistant phage growth and a drug-resistant viral topoisomerase, we conclude that the T4-encoded type II DNA topoisomerase is the physiological target of *m*-AMSA in phage-infected *E. coli*.

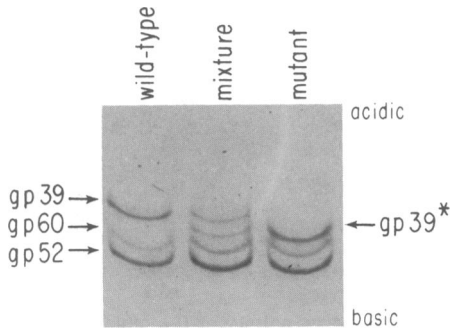

FIG. 4. Mutant topoisomerase contains an altered gene *39* subunit. Samples of the wild-type enzyme (0.7 μg), mutant enzyme (0.7 μg), and a mixture of the two (0.7 μg each) were analyzed by denaturing nonequilibrium pH-gradient gel electrophoresis. A photograph of the Coomassie brilliant blue R-250-stained gel is shown. Subunits were assigned by excising all bands from the pH-gradient gel and subjecting them to NaDodSO$_4$/PAGE (also see ref. 33); the mutationally altered subunit is indicated by an asterisk (gp39*).

The location of the drug-resistance mutation in gene *39* rather than gene *52* is surprising in view of the reported roles of the individual topoisomerase subunits. Gp39 apparently contains the active site for ATP hydrolysis (34), while gp52 is directly involved in DNA cleavage/resealing (16). Thus, even though *m*-AMSA induces DNA cleavage by the T4 topoisomerase, a mutation in the ATPase subunit can confer drug resistance. This differs from earlier results with bacterial DNA gyrase, in which mutational alteration of the DNA cleavage/resealing subunit was shown to confer resistance to nalidixic acid (an antibacterial drug that induces gyrase-mediated DNA cleavage) (35, 36). However, nalidixic acid-resistance mutations in the ATPase subunit of *E. coli* DNA gyrase have also been reported (37).

The *m*-AMSA^R mutant T4 topoisomerase exhibited three unexpected differences from the wild-type enzyme: (*i*) decreased specific activity for DNA relaxation, (*ii*) enhanced DNA cleavage in the absence of *m*-AMSA, and (*iii*) altered DNA cleavage site specificity. The most intriguing aspect of these properties is that each mimics an effect that the inhibitor *m*-AMSA has on the wild-type enzyme. Both the drug-resistance mutation and *m*-AMSA reduce DNA relaxation activity, enhance DNA cleavage, and alter the DNA cleavage-site specificity; the inhibitor is quantitatively more effective at producing the first two effects. How could a mutation that blocks drug action partially mimic the effects of the drug? One model for topoisomerase inhibition is that *m*-AMSA binds avidly to some site on the covalent topoisomerase–DNA complex (reaction intermediate), thereby stabilizing the complex and blocking the resealing step of the reaction cycle (see above). Perhaps the *m*-AMSA^R mutation confers resistance by directing the insertion of an amino acid side chain into the drug-binding site. This insertion at the drug-binding site may then disrupt the topoisomerase reaction cycle in the same qualitative (but not quantitative) manner as insertion of a drug molecule at that site.

The altered properties of the drug-resistant T4 topoisomerase are reminiscent of those of a mammalian DNA topoisomerase I resistant to camptothecin [an antitumor agent that induces DNA cleavage by topoisomerase I (30)]. The camptothecin-resistant topoisomerase I participates in covalent enzyme-DNA complexes that exhibit increased kinetic stability and salt resistance (38). The decreased specific activity and enhanced DNA cleavage of the *m*-AMSA^R T4 topoisomerase can likewise be explained by an increased stability of the reaction intermediate for the mutant enzyme.

Because a variety of topoisomerase inhibitors induce the accumulation of covalent complexes with different topoisomerases (see above), there may exist a unified molecular mechanism of drug action. It might be expected that topoisomerase inhibitors bind directly to the enzyme, with drug resistance conferred by mutations that block drug binding. However, binding studies did not detect any direct interaction between a nalidixic acid analog and DNA gyrase, but instead indicated that the drug binds DNA (39). Many antitumor agents that block the mammalian type II topoisomerase are intercalating agents, again suggesting that drug binding to DNA may be important (40). As described above, one general model proposes that a ternary drug–DNA–enzyme complex is the relevant structure in the mechanism of inhibition (3). A second model is that drug-induced alterations in DNA structure disrupt the topoisomerase reaction cycle; no direct interaction between drug and topoisomerase would be required. The alteration of DNA cleavage site specificity both by *m*-AMSA and by the

m-AMSA^R mutation (Fig. 3) is consistent with either of these two general models. The rules of DNA site recognition used both by topoisomerases and by inhibitors (e.g., *m*-AMSA) will need to be carefully considered in deducing the molecular mechanism of topoisomerase inhibition (15, 41–43).

In summary, we have shown that the T4-encoded type II DNA topoisomerase is the physiological target of *m*-AMSA in T4-infected *E. coli*. This assignment is further substantiated by the finding that T4 topoisomerase is involved in the mechanism of *m*-AMSA-induced frameshift mutagenesis in T4-infected *E. coli* (44). These results, coupled with highly suggestive evidence from mammalian systems, strongly support the proposal that the type II DNA topoisomerase is also the specific intracellular target of antitumor drug action in mammalian cells. Further characterization of the wild-type and drug-resistant T4 topoisomerases should facilitate comparable studies in mammalian systems and permit the development of a refined molecular model for the sensitivity and resistance of type II topoisomerases to antitumor agents.

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