Mutation adjacent to the active site tyrosine can enhance DNA cleavage and cell killing by the TOPRIM Gly to Ser mutant of bacterial topoisomerase I

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ABSTRACT

The TOPRIM DXDXXG residues of type IA and II topoisomerases are involved in Mg(II) binding and the cleavage-rejoining of DNA. Mutation of the strictly conserved glycine to serine in Yersinia pestis and Escherichia coli topoisomerase I results in bacterial cell killing due to inhibition of DNA religation after DNA cleavage. In this study, all other substitutions at the TOPRIM glycine of Y. pestis topoisomerase I were examined. While the Gly to Ala substitution allowed both DNA cleavage and religation, other mutations abolished DNA cleavage. DNA cleavage activity retained by the Gly to Ser mutant could be significantly enhanced by a second mutation of the methionine residue adjacent to the active site tyrosine. Induction of mutant topoisomerase with both the TOPRIM glycine and active site region methionine mutations resulted in up to 40-fold higher cell killing rate when compared with the single TOPRIM Gly to Ser mutant. Bacterial type IA topoisomerases are potential targets for discovery of novel antibiotics. These results suggest that compounds that interact simultaneously with the TOPRIM motif and the molecular surface around the active site tyrosine could be highly efficient topoisomerase poisons through both enhancement of DNA cleavage and inhibition of DNA rejoining.

INTRODUCTION

DNA topoisomerases can carry out the important functions of DNA supercoiling regulation and DNA untangling because these enzymes can catalyze the interconversion of DNA topological forms by the concerted breaking and rejoining of DNA strands coupled to DNA strand passage through the DNA cleavage sites (1–4). A covalent enzyme–DNA complex is formed after nucleophilic attack of an active site tyrosine on the DNA phosphodiester backbone during the DNA cleavage step. Topoisomerase poisons are compounds that interfere with the DNA cleavage-rejoining equilibrium of topoisomerases and increase the physiological concentration or life-time of the covalent complex intermediates formed between topoisomerases and cleaved DNA, resulting in events that lead to cell death (5,6). Many clinically important antibacterial and anticancer drugs are topoisomerase poisons targeting type IB and type IIA topoisomerases (5–10). Both type IA and type IB topoisomerases cut and rejoin a single-strand of DNA during catalysis but type IA topoisomerases are more similar to type IIA topoisomerases in catalytic mechanism of DNA cleavage and rejoining (3).

Based on genome sequences and genetic studies, there is at least one type IA topoisomerase activity required to be present in every organism to resolve entanglement of single-stranded DNA during replication or recombination (2,11). Although topoisomerase poison known to target type IA topoisomerase with high specificity is not available currently, it has been demonstrated that trapping of covalent complex intermediate formed by a mutant form of recombinant Yersinia pestis or Escherichia coli topoisomerase I can result in rapid and extensive cell killing in E. coli (12). This cell killing effect from the stabilization of topoisomerase I cleavage complex validates the potential of bacterial type IA topoisomerase as a target for development of novel antibacterial compounds to combat multi-drug resistant bacterial pathogens (12–14). The cell killing mutation previously characterized was identified by screening a random recombinant Y. pestis topoisomerase I mutant library for induction of cellular SOS response in E. coli, and involves change of the strictly conserved Gly residue in the TOPRIM motif (DXDXXG) to a Ser residue (12). The understanding of how DNA cleavage-rejoining can be interfered with at the...
molecular level is important for the elucidation of how the enzyme maintains the DNA cleavage-rejoining equilibrium so that the degree of DNA cleavage is sufficient for the required conversion of DNA topology, but avoids toxic level of cleaved DNA complex being accumulated. Such biochemical investigations could also be useful for the design of novel type IA topoisomerase poisons or modification of hit molecules identified via high-throughput screening in the development of antibacterial compounds targeting type IA topoisomerases (13,14). In this study, it is shown that the TOPRIM Gly to Ser mutation in subdomain I is unique in its cell killing capability among all other possible substitutions at the conserved Gly residue because it maintains a sufficient level of DNA cleavage activity while DNA religation is abolished. All other possible amino acid substitutions at the conserved glycine residue of the TOPRIM motif were examined and found not to result in cell killing. Every other substitution except Ala had no detectable DNA cleavage or relaxation activity. Unlike the Gly to Ser substitution, DNA religation was not inhibited by the Gly to Ala substitution. We also demonstrated that in a different subdomain of the enzyme (subdomain III), although the conversion of the highly conserved methionine residue adjacent to the active site tyrosine of Y. pestis topoisomerase I did not inhibit relaxation of DNA or affect cell viability by itself, the mutation enhanced DNA cleavage by the enzyme. When this increase in DNA cleavage was combined with the inhibition of DNA religation by the TOPRIM Gly to Ser mutation, the level of DNA cleavage product and degree of cell killing of the double mutant were increased significantly over the TOPRIM Gly to Ser single mutant. This demonstrates that molecular interactions near the active site tyrosine of type IA topoisomerases can also affect the DNA cleavage-religation equilibrium and be synergistic with other molecular perturbations elsewhere in the enzyme that inhibit DNA religation to achieve higher degree of DNA cleavage product accumulation and overall bactericidal action.

MATERIALS AND METHODS

Site-directed mutagenesis of recombinant topoisomerase I

Site-directed mutagenesis was carried out with using Pfu Ultra DNA polymerase (from Stratagene) with procedures based on the Stratagene QuikChange protocol. Oligonucleotide primers coding for the desired amino acid substitutions were synthesized by Sigma Genosys. Random substitution of Y. pestis topoisomerase I (YTOP) at Gly122 was achieved with plasmid pYTOP (12) as template and primers 5’GACCTTGATCGCGAA NNNAGGGCTATTTGCCGT 3’ and 5’ CAGGCAATAG CCTCNNTTGGCGATCAAGGTC 3’. YTOP mutants with substitutions of Gly122 with Asn, His, Trp and Tyr or Met326 substitution with Val were created by using oligonucleotides specifying these substitutions. Recombinant E. coli topoisomerase I (ETOP) with Val substitution at Met320 were made by introducing the site-directed mutation into plasmid pETOP or pETOP-G116S (12). In the pYTOP and pETOP plasmids, the synthesis of the recombinant topoisomerase I is under the control of the arabinose-inducible BAD promoter. In addition to ampicillin, the plates and media used for isolation, maintenance and overnight growth of the E. coli strains transformed with plasmids pYTOP or pETOP contained 2% glucose to suppress the expression of the potentially lethal mutant topoisomerases from the BAD promoter.

Identification of SOS inducing YTOP mutants

Escherichia coli strain JD5 with chromosomal dinD1::lacZ fusion (15) was transformed with plasmid pYTOP derivatives encoding mutant YTOP enzymes. Transformations obtained on LB plates with 2% glucose and 100 μg/ml ampicillin were replicated onto plates with ampicillin, 35 μg/ml X-gal and 0.002% arabinose to identify SOS-inducing mutants that gave rise to blue colonies after overnight incubation at 37°C due to induction of the dinD1 promoter (16).

Effect of recombinant topoisomerase expression on viability

The ability of the YTOP mutants with different substitutions at Gly122 to cause bacterial cell death was first evaluated in strain JD5 by inducing their expression with saturating concentration of arabinose (0.2%) for 2 h at early exponential phase (OD₆₀₀ = 0.4) after dilution of overnight culture in the presence of 2% glucose into fresh LB medium with antibiotics but no glucose. Viable counts were measured by plating of dilutions on LB plates with 2% glucose and ampicillin and compared with viable counts from cultures not induced with arabinose after overnight incubation of the plates at 37°C. The cell killing effects of mutant recombinant topoisomerases with the Gly to Ser substitution were also measured at a range of lower arabinose concentrations (0.0006–0.002%) in E. coli strain BW27784 (from Yale E. coli Genetic Stock Center). In this strain, the control of the arabinose transporter araE gene by a constitutive promoter (17) allows expression from the BAD promoter to be regulated by increasing arabinose concentration instead of the ‘all-or-none’ expression pattern found in strain JD5 (12,18). This enabled comparison of the cell killing efficiency with increasing arabinose concentrations.

Protein purification

Wild-type and mutant YTOP proteins were induced in E. coli JD5 strain after growth in LB medium with ampicillin to exponential phase with either 0.02 or 0.2% arabinose for 4 h at 37°C. The recombinant proteins with thioredoxin N-terminal tag and His₆ C-terminal tag were purified with the His SpinTrap column (GE Healthcare) according to manufacturer’s protocol. Recombinant ETOP mutant proteins without affinity tags were expressed in E. coli strain GP200 (Atop4) and purified by combination of phosphocellulose, hydroxypatite and DNA affinity chromatography columns as previously described (19,20).
Enzyme activity assays

Each assay was carried out at least thrice, and representative results are shown here. Assays of relaxation of negatively supercoiled DNA by topoisomerase I was assayed in the presence of 6 mM MgCl₂ as described previously (20). Cleavage of plasmid DNA was assayed in buffer with either no added Mg²⁺ or with up to 10 mM MgCl₂ added as described (12). A 59-base oligonucleotide 5′-GCCCTGAAAGATTATGCATGCGCTTTGGGC AAACCAAGAGCTAATCTTTCAGGGC-3′ with the preferred cleavage site CAAT↓GC for ETOPO (21) was labeled at the 5′-end with T4 polynucleotide kinase and [γ³²P-ATP]. Cleavage and religation of the oligonucleotide by topoisomerase I were assayed as described and analyzed by electrophoresis in a 15% DNA sequencing gel (22). The wild-type topoisomerase I religation reaction is complete after 10 s at 37°C so some of the religation reactions were carried out on ice at 0°C. Gel shift assay with the same 5′-end labeled oligonucleotide was used to compare non-covalent binding affinities of wild-type and mutant ETOPO enzymes with the DNA substrate (22). To assess the effect of the mutations on Mg²⁺-binding affinity of ETOPO, measurements of change in intrinsic tryptophan fluorescence of ETOPO enzymes from binding of Mg²⁺ (20,23) were carried out as described previously (12). Non-linear regression curve fitting for two binding sites was carried out using the GraphPad Prism program.

RESULTS

Only Ser substitution at Gly122 of YTOP produced the SOS inducing and cell killing phenotypes by inhibiting DNA religation while retaining DNA cleavage activity

To determine if in addition to substitution with Ser, other types of amino acid substitutions at Gly122 of *Y. pestis* topoisomerase I can have SOS inducing and cell killing properties, random mutations at Gly122 were first created by oligonucleotide-directed mutagenesis with all four possible nucleotides at the codon positions for Gly122 of recombinant YTOP expressed under the tight control of the BAD promoter. After sequencing 60 mutants with random substitutions at Gly122 and screening for SOS induction using the *dinD1::lacZ* reporter strain JD5, only the Ser substitution mutants were found to have the SOS-inducing phenotype on X-gal plates with low level of arabinose (0.002%). Gly122 substitutions to Asn, His, Trp and Tyr were the only possible substitutions not found among the 60 sequenced YTOP mutants. These substitution mutants were then created by sequence specific oligonucleotide-directed mutagenesis. These four mutants were also found not to induce SOS in strain JD5. The effect of overexpression of 13 of the YTOP Gly122 substitution mutants on cell viability in strain JD5 were measured by determination of viable counts at 2 h after induction with saturating concentration of arabinose (0.2%). The list of mutants analyzed for effect on viability included those substitutions most similar to Gly and Ser in size or having the same functional group in the side chain (Thr and Tyr), as well as some examples of substitutions that are much larger in size and hydrophobic in nature. The results (Table 1) confirmed that these YTOP Gly122 substitution mutants that did not induce the SOS DNA damage response also had relatively small effect on cell viability when compared with the Gly122 to Ser substitution YTOP mutant. The substitution with cysteine had a greater effect on viability (induced/non-induced relative viability = 0.03) than the other substitutions examined (average relative viability = 0.11), but the relative viability of the cysteine substitution is still 100-fold higher than that obtained for the serine substitution.

Effect of the Gly122 substitutions on YTOP enzyme activity

The YTOP mutants with every possible Gly122 substitutions were purified for assay of relaxation activity and DNA cleavage activity. The expression level of each of the mutant proteins in the soluble extract of *E. coli* JD5 was similar to that of the wild-type YTOP protein. The G122A mutant YTOP was found to retain around 10% of the relaxation activity (Figure 1A). All the other substitution mutants, similar to the G122S mutant (12), had no detectable relaxation activity (data not shown). DNA cleavage activity was assayed both in the absence and presence of up to 10 mM MgCl₂ in the reaction. The addition of Mg²⁺ is not necessary for the DNA cleavage activity of *E. coli* and *Y. pestis* topoisomerase I to be reduced and it was necessary to have Mg²⁺ added to the reaction mixture to observe DNA cleavage (12). The YTOP-G122A mutant enzyme was also dependent for DNA cleavage activity and had no detectable DNA cleavage activity in the absence of added Mg²⁺ (Figure 1B). For the wild-type YTOP enzyme, addition of MgCl₂ promotes religation, so the amount of cleavage product

| Substitution at Gly122 | Induced/non-induced relative viability |
|-----------------------|---------------------------------------|
| none                  | 0.17 ± 0.11                           |
| Ser                   | 5.2 × 10⁻⁷ ± 1.8 × 10⁻⁵               |
| Ala                   | 0.093 ± 0.015                         |
| Asp                   | 0.19 ± 0.02                           |
| Asn                   | 0.17 ± 0.10                           |
| Cys                   | 0.030 ± 0.004                         |
| Glu                   | 0.092 ± 0.040                         |
| Gln                   | 0.11 ± 0.06                           |
| His                   | 0.14 ± 0.02                           |
| Ile                   | 0.086 ± 0.033                         |
| Phe                   | 0.075 ± 0.035                         |
| Thr                   | 0.087 ± 0.018                         |
| Trp                   | 0.13 ± 0.05                           |
| Tyr                   | 0.088 ± 0.016                         |

Relative viability (RV) was measured by the ratio of the viable colonies obtained after induction of the BAD promoter directing the expression of recombinant YTOP with 0.2% arabinose for 2 h in comparison with viable colonies from the culture not treated with arabinose. The results shown represent the average and standard deviation from at least three measurements.

Table 1. Effect of overexpression of recombinant wild-type or mutant *Y. pestis* topoisomerase I with substitutions at Gly122 on the viability of *E. coli* JD5

For the wild-type YTOP enzyme, addition of MgCl₂ promotes religation, so the amount of cleavage product
in the presence of 2 mM MgCl$_2$ was reduced by 56% (from densitometry analysis of four sets of data) when compared with that observed in the absence of Mg$^{2+}$ (Figure 1B, left panel). In the presence of 2 mM MgCl$_2$, the amount of cleavage product formed by the G122A mutant was about the same as the wild-type enzyme (122% from three sets of data). While the YTOP-G122S mutant enzyme has no detectable DNA rejoining activity (12), YTOP-G122A mutant enzyme was found to be capable of DNA rejoining (Figure 1C). For both wild-type YTOP and YTOP-G122A, rejoining of cleavage product reached >90% of the maximal level within 30 s after the addition of MgCl$_2$ and high salt at 37°C.

DNA cleavage was not observed for any of the other YTOP G122 substitution mutant protein in the absence of added Mg$^{2+}$ and the addition of up to 10 mM Mg$^{2+}$ did not restore the DNA cleavage activity of any of the other Gly122 substitution mutants (data not shown). Due to its more significant effect on viability among the mutants examined, DNA cleavage activity of YTOP-G122C was also examined using 5'-end labeled oligonucleotide as substrate. DNA cleavage activity was again not detectable, so the small effect on viability from overexpression of YTOP-G122C was probably unrelated to the effect of the mutation on DNA cleavage-religation. It can be concluded that the Gly to Ser substitution is unique in its cell killing phenotype because it is the only amino acid substitution at this critical position that inhibits DNA religation completely while retaining a high degree of DNA cleavage activity.

Mutation of the conserved Met residue adjacent to the active site tyrosine to Val can enhance the cell killing activity of the TOPRIM Gly to Ser topoisomerase I mutant

The sequence of the SOS-inducing YTOP128 mutant isolated in the original mutant screening had two other mutations, M326V and A383P, in addition to the TOPRIM G122S mutation (12). Site-directed mutagenesis showed YTOP with single M326V or A383P mutation had no SOS-inducing or significant cell killing effect in strain JD5 (12). The cell killing effect from YTOP-G122S was slightly lower than the original YTOP128 mutant in strain JD5 (12). Met326 follows the active site tyrosine residue Tyr325 in YTOP sequence and is conserved in bacterial topoisomerase I sequences, while a proline is often found in other type IA topoisomerases at this position (26). To determine the effect of the additional M326V mutation on the cell killing efficiency of YTOP-G122S, the double mutant YTOPG122S/M326V was created by site-directed mutagenesis on the low copy number expression plasmid pAYTOP derived from the cloning vector pACYC184 (14). In the genetic background of JD5, arabinose induction of the BAD promoter is ‘all or none’ because the araC-P$_{BAD}$ system and the associated L-arabinose transporter AraE are regulated autocatalytically by arabinose (18). The experiments in JD5 therefore utilized the saturating concentration of arabinose (0.2%) for measurement of cell viability. In strain BW27784, the synthesis of AraE is arabinose independent due to its control under a constitutive promoter (17), allowing increasing amount of YTOP proteins to be expressed from the BAD promoter by increasing concentrations of arabinose added to the culture. Strain BW27784 was used to compare the cell killing efficiency of the low copy number plasmid pAYTOP expressing wild-type YTOP, and its derivatives expressing YTOP-G122S or YTOP-G122S/M326V, as well as the original YTOP128 mutant over a range of non-saturating concentrations of arabinose (Table 2). The results demonstrated that the addition of the M326V mutation to the YTOP-G122S mutant
The Met to Val substitution adjacent to the active site tyrosine enhanced DNA cleavage

The effect of the substitution of Met326 with Val on YTOP protein activity was further analyzed to determine the biochemical basis for this enhancement of cell killing. When the enzyme activity of YTOP-M326V was examined, it was found to have no significant effect on the relaxation activity when compared with wild-type YTOP enzyme (Figure 2A). Although YTOP-M326V had no SOS-inducing or cell killing effect in vivo (12), the amount of cleavage product formed by this mutant was higher than that from wild-type YTOP (Figure 2B). Densitometry analysis of results from three experiments showed that the Met to Val substitution resulted in 1.9-fold higher cleavage product than wild-type YTOP in the absence of Mg2+. However, the percent of decrease of cleavage product upon addition of 2 mM MgCl2 was higher for YTOP-M326V (76%) than wild-type YTOP (53%), so the amount of cleavage product was approximately equal for YTOP-M326V and wild-type YTOP at 2 mM MgCl2. This is in good agreement with the lack of SOS induction or cell killing by the YTOP-M326V mutant in vivo with Mg2+ present.

To study the effect of this mutation on the enzymatic activity of type IA topoisomerases with the better characterized system of ETOP, the corresponding M320V and M320V/G116S ETOP mutant enzymes were expressed from the BAD promoter in plasmid pETOP (12). These mutant enzymes without any linked affinity tags were purified by a combination of conventional chromatography procedures and compared with wild-type ETOP and the ETOP-G116S mutant enzyme characterized previously (12). Similar to YTOP-M326V enzyme, ETOP-M320V protein had wild-type relaxation activity (Figure 3A), but enhanced DNA cleavage activity (Figure 3B and C). Densitometry analysis of the cleavage products from three sets of data showed that the level of cleavage product formed by the M320V mutant enzyme was 2.2-fold that of the wild-type ETOP in the absence of Mg2+. Similar to wild-type YTOP, the presence of 2 mM or higher MgCl2 promoted religation by ETOP, so there was a decrease in the cleavage products formed with both wild-type and M320V ETOP upon addition of MgCl2. Religation of cleavage product in the presence of MgCl2 and high salt was complete within 10 s at 37°C, so the religation reaction was carried out on ice at 0°C. At this temperature,
of the double mutant ETO-G116S/M320V enzyme were strongly inhibited (Figure 4A and D), as expected from the known effect of the G116S mutation on ETO-P from previous study (12). DNA cleavage activity was Mg\(^{2+}\) dependent as in the case of the ETO-G116S mutant (Figure 4B), and the level of DNA cleavage product formed by the ETO-G116S/M320V mutant was increased over the ETO-G116S mutant by \(~3\)-fold (Figure 4C). The increase in DNA cleavage provided a basis for the enhancement of cell killing \(in vivo\) when the Met to Val mutation in subdomain III was combined with the subdomain I TOPRIM Gly to Ser mutation.

### DISCUSSIONS

In this study, it was determined that the Ser substitution at the strictly conserved Gly residue of the topoisomerase I TOPRIM motif was unique in its consequence of inhibiting DNA religation while retaining DNA cleavage activity, thus accounting for an effect on enzyme activity and cell viability similar to that expected from a
topoisomerase poison. Among all amino acids, glycine has the smallest and most flexible side chain. The size of the side chain increases in the order of Gly to Ala to Ser. When the TOPRIM Gly was substituted with Ala, Mg²⁺-binding affinity was likely to have also been affected, as DNA cleavage became Mg²⁺ dependent as in the case of the Gly to Ser substitution. However, the Ala substitution might not have altered the positioning of the DNA 3'-hydroxyl group formed after DNA cleavage as much as the Ser substitution to have an inhibitory effect on DNA religation. With all the other 17 amino acid substitutions tested here, the steric effect from replacing the strictly conserved Gly side chain with a much larger group probably accounted for the complete loss of DNA cleavage activity, even in the presence of up to 5 mM Mg²⁺. The strict requirement of Gly for its steric properties probably accounts for its high degree of conservation following the DXD residues in the TOPRIM domain of DNA topoisomerases (26,27). The results from these experiments illustrate how the two events of DNA cleavage and DNA religation in the catalytic cycle, can be differentially affected by molecular perturbations even though the DNA religation is the reverse of DNA cleavage step when considered only for overall chemical changes. Small molecules could potentially bind adjacent to the Gly residue of the topoisomerase IA TOPRIM motif in

Figure 4. Effect of addition of the M320V mutation to the ETOP-G116S mutant enzyme activities. (A) Restoration of a low level of relaxation activity to the ETOP-G116S mutant enzyme by the M320V mutation. The indicated amount of enzyme was incubated with supercoiled plasmid DNA in buffer containing 6 mM MgCl₂ at 37 °C for 30 min. S: supercoiled plasmid DNA. (B) Dependence of magnesium for DNA cleavage. 400 ng of enzyme was incubated with 0.5 pmoles of substrate for 30 min at 37 °C. (C) Quantitation of oligonucleotide (5 pmoles) cleavage by ETOP-G116S (filled circles) and ETOP-G116S/M320V (open circles) enzymes in the presence of 5 mM MgCl₂. The data plotted represents the average and standard deviation from three different experiments. (D) DNA religation inhibition for the ETOP-G116 and ETOP-G116S/M320V mutants. The oligonucleotide substrate was incubated with 400 ng of enzyme in the presence of 5 mM MgCl₂ at 37 °C for 10 min at 37 °C before the addition of 1 M NaCl and further incubation at 37 °C for the indicated length of time.
subdomain I and exert effect selectively on the DNA
relication step while allowing the enzyme to cleave DNA
and form a covalent complex, similar to the effect of the
Ser substitution.

Single mutation of the Met residue adjacent to the
active site Tyr to Val was found not to have SOS-inducing
or cell killing phenotypes in vivo, but nevertheless resulted
in increase in DNA cleavage activity in vitro. There have
been no mutagenesis results on this residue until this
study. The Met to Val is a relatively conservative substi-
tution so it is not surprising that the substitution did not
inhibit the relaxation activity. Nevertheless, substitutions
between Met and Val in other proteins have been shown
to have significant effects on protein folding or catalytic
properties (28–30). This result demonstrated for the first
time that perturbation proximal to the active site tyrosine
in subdomain III of topoisomerase I could influence DNA
cleavage efficiency.

When this second Met to Val mutation adjacent to
the active site tyrosine was added to the YTOP-G122S or
ETOP-G116S TOPRIM mutants, DNA cleavage activity
was enhanced while DNA rejoining was still inhibited
effectively by the steric effect of the Gly to Ser substitution
in subdomain I, resulting in a higher level of bacterial cell
killing when the double mutant enzyme was induced by
arabinose in vivo. This biochemical study showed that a
perturbation in subdomain I of type IA topoisomerase
that inhibits DNA relication can be combined with a
second perturbation in subdomain III that enhances DNA
cleavage in the process of ‘poisoning’ the topoisomerase
enzyme. It might be possible to achieve the goal of
creating a type IA topoisomerase poison by combining
two small molecule fragments that interact separately with
the TOPRIM motif and the region adjacent to the active
site tyrosine (Figure 6). Bacterial type IA topoisomerases
could potentially be a useful new target for discovery of
novel antibacterial compounds to combat the serious
public health problem of rapidly increasing instances of
bacterial pathogens resistant to the antibacterial therapy
currently in use (31–34).

High throughput screening of compound libraries is
one approach that is being utilized in the attempt of iden-
tifying small molecules that can act as bactericidal poisons
and increase the accumulation of covalent complex
formed by bacterial type IA topoisomerases and cleaved
DNA (14). The identification of various regions of the
enzyme structure and associated molecular perturbations
that can influence the DNA cleavage-religation equili-
brum of topoisomerase I could facilitate a second alter-
native approach of drug discovery through molecular
modeling (35–37). For such molecular modeling efforts,
it would be extremely useful to have a crystal structure of
the covalent complex formed between a type IA topo-

isomerase and cleaved DNA available. Structure of the
covalent complex would also complement the currently
available 3-dimensional structures of the non-covalent
complexes in different conformational states formed
between type IA topoisomerases and cleaved DNA (38–40)
to provide key information on the catalytic mechanism and
protein conformational changes that take place during the
catalytic cycle. The mutant enzyme molecules that have
been identified in our ongoing study as those that could
form stabilized covalent intermediate with cleaved DNA
might be more amenable to efforts of obtaining a crystal
structure of the covalent complex between type IA
topoisorase and cleaved DNA substrate.
SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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