On the Molecular Basis of the Thermal Sensitivity of an
Escherichia coli topA Mutant*

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Studies of two temperature-sensitive Escherichia coli topA strains AS17 and BR83, both of which were supposed to carry a topA amber mutation and a temperature-sensitive supD3,74 amber-suppressor, led to conflicting results regarding the essentiality of DNA topoisomerase I in cells grown in media of low osmolality. We have therefore reexamined the molecular basis of the temperature sensitivity of strain AS17. We find that the supD allele in this strain had lost its temperature sensitivity. The temperature sensitivity of the strain, in media of all osmolality, results from the synthesis of a mutant DNA topoisomerase I that is itself temperature-sensitive. Nucleotide sequencing of the AS17 topA allele and studies of its expected cellular product show that the mutant enzyme is not as active as its wild-type parent even at 30 °C, a permissive temperature for the strain, and its activity relative to the wild-type enzyme is further reduced at 42 °C, a nonpermissive temperature. Our results thus implicate an indispensable role of DNA topoisomerase I in E. coli cells grown in media of any osmolality.

Bacterial DNA topoisomerase I is a member of the type IA subfamily of DNA topoisomerases found in all living organisms (reviewed in Refs. 1–3). The enzyme was first identified in Escherichia coli three decades ago as the “omega protein” (4), and its structural gene topA was mapped and sequenced in the 1980s (5–7). Extensive mechanistic studies of the enzyme and its mutants have been carried out (1–3), and the crystal structure of a 67-kDa fragment of it was reported in 1994 (8). In the three-dimensional structure, the polypeptide chain folds into a domain containing a catalytic site that possesses a unique active site architecture (9). The architecture was seen in the crystal structure of E. coli DNA topoisomerase III (9), another member of the type IA subfamily.

E. coli DNA topoisomerase I specifically relaxes negatively supercoiled DNA (4) and has a key role in the modulation of intracellular DNA supercoiling (1–3). Inactivation of the enzyme is detrimental to cell viability (10–12). Excessive negative supercoiling of intracellular DNA, particularly in regions behind the transcribing RNA polymerases (13), appears to be a major cause of lethality of E. coli topA null mutants (1–3). In support of this notion, secondary mutations that reduce the cellular level of gyrase (DNA topoisomerase II), an activity that catalyzes DNA negative supercoiling or the removal of positive supercoils, was found to suppress topA lethality (10, 11). Furthermore, expression of eukaryotic DNA topoisomerase I (14) or vaccinia virus topoisomerase (15), as well as overexpression of E. coli DNA topoisomerase III (16) or IV (17, 18), was also found to compensate for topA inactivation. One consequence of excessive negative supercoiling of intracellular DNA appears to be a hybrid formation between nascent RNA and the DNA template (“R-looping”), as suggested by the partial suppression of topA lethality by overexpressing RNaseH (19, 20). Unlike E. coli, Salmonella typhimurium and Shigella flexneri topA nulls appear to survive in the presence of a compensatory mutation (21, 22).

In addition to its role in the regulation of DNA supercoiling, bacterial DNA topoisomerase I is likely to participate in other processes that require the passage of one DNA single strand through an enzyme-mediated transient break in another. E. coli topA topB double mutants lacking both DNA topoisomerase I and III are nonviable even in the presence of a mutation that compensates for topA inactivation (23, 24).

The involvement of bacterial DNA topoisomerase I in multiple cellular transactions of DNA is manifested by a pleiotropic phenotype of topA mutants. In addition to their effects on E. coli cell lethality (10–12), mutations in topA were also reported to affect adaptive responses to changes in environmental conditions (reviewed in Refs. 25, 26; see also Refs. 27–30), plasmid partition (31, 32), chromosome segregation in E. coli muk mutants (33), the development of genetic competence in Hemophilus influenza (34), sensitivity of Salmonella typhimurium to ultraviolet irradiation (35, 36), and recA-independent recombination (37).

Because of the multiple cellular roles of bacterial DNA topoisomerase I, conditional topA mutants were constructed to facilitate functional studies of this enzyme. In one strain, the coding sequence of topA was placed under the control of a lac promoter, so that expression of the gene could be tightly regulated (38). Temperature-dependent expression of E. coli topA was also accomplished in strains AS17 and BR83, which were constructed by the introduction of an amber mutation in topA and the expression of a plasmid-borne or chromosomally located temperature-sensitive (ts) amber-suppressor (R. E. Depew, in Refs. 39, 40). The isolation of ts alleles within topA has been unsuccessful; introducing a plasmid-borne topA ts mutation C662H (41) into the chromosomal topA gene, for example, yielded a mutant that grew well at 30 or 42 °C.2

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1 The abbreviation used is: ts, temperature-sensitive.
2 V. Stupina, Y. Wang, and J. C. Wang, unpublished data.
Because of the lack of known ts alleles within topA, the thermal-sensitive topA strains AS17 and BR83 were used in a number of previous studies (see for examples, Ref. 14, 39–43).

In the course of working with these strains, however, we encountered several observations that could not be explained by a common molecular basis of the temperature sensitivity of these strains, namely the temperature-dependent synthesis of a functional DNA topoisomerase I. We report here that the plasmid-borne supD amber suppressor in strain AS17 had apparently lost its temperature sensitivity. Both in vivo and in vitro experiments provided strong evidence that the ts phenotype of strain AS17 resulted from the synthesis of a temperature-sensitive DNA topoisomerase I in the presence of a functional supD, and not from the temperature-dependent suppression of an amber codon in the topA allele of the strain. Our results also indicate that the growth of E. coli cells is critically dependent on the presence of a functional DNA topoisomerase I in media of any osmolarity. Previously, studies of BR83 cells grown in different media had led to the suggestion that the enzyme might be dispensable in growth media of low osmolarity (40).

**EXPERIMENTAL PROCEDURES**

Identification of Mutations in the topA Gene of Strain AS17—The topA region of DNA from strain AS17 (F topA17/am) pLL1(Tet supD4347)) was amplified by the polymerase chain reaction (PCR), using a pair of primers 5′-ATG-GGT-GAT-CTG-GAG-CTG-GTT-GCC-ACT-GAT-GGA-AGG-TTT-3′ and 5′-GGC-ATC-TAG-ATG-AGA-CGA-CTA-TAT-CAT-TTA-TAG-CCT-3′. Each of the primers incorporated a unique restriction site (underlined) to facilitate subsequent cloning of the PCR product. The 2.8-kb XhoI-XbaI fragment containing the entire coding region of topA was purified by gel electrophoresis from the restriction endonuclease-treated PCR products and subcloned. The topA insert in the subcloned plasmids was sequenced (carried out at the Molecular Biology Core Facility of the Medical College of Georgia, Augusta, Georgia).

Site-directed Mutagenesis—Site-directed mutagenesis using a commercial kit (Stratagene) was done to introduce specific mutations into the topA region of pJW312, a plasmid previously constructed for the overexpression of wild-type topA from a lac promoter (39). Two pairs of mutagenic oligonucleotides, 5′-CT-AAA-GAT-GAA-CAG-GCG-CAC-GTC-3′ and 5′-GGC-ATC-TAG-ATG-AGA-CGA-CTA-TAT-CAT-TTA-TAG-CCT-3′, and their complements were used to introduce the G65N or the W79S mutation. The double mutant harboring both G65N and W79S mutations was constructed by two successive rounds of site-directed mutagenesis. Further confirmation of the presence of the intended mutation or mutations was carried out by nucleotide sequencing.

In Vivo Complementation Assay—Plasmids pJW312 and its derivatives bearing the specified mutations, pJW312(G65N), pJW312(W79S), pJW312(G65N/W79S), and pJW312(Y319A), were first digested with BalIII and HindIII. The 2.9-kb fragment containing the lac promoter-linked wild-type or mutated topA gene was then inserted in between the BalIII and HindIII sites of a single-copy plasmid pBeloBAC11 (purchased from New England Biolabs). The resulting constructs were individually transformed into AS17 cells, and the transformants were checked for growth at different temperatures on Luria broth agar plates containing tetracycline and chloramphenicol. Plasmids expressing wild-type E. coli DNA topoisomerase I and the active site tyrosine mutant (Y319A) protein were included in this experiment as the positive and negative controls, respectively.

Overexpression and Purification of Wild-type and Mutant E. coli DNA Topoisomerase I—pJW312 and pJW312(G65N/W79S) were individually transformed into a ΔtopA E. coli strain DM800 bearing a pACYC184-based plasmid overexpressing the lac repressor (44). The use of a ΔtopA strain as the host eliminates the possible contamination of wild-type DNA topoisomerase I in the preparation of the mutant protein. Induction of cells for overexpression of the wild-type and mutant enzymes was performed at 30 °C by the addition of isopropyl-1-thio-β-D-galactoside to 1 mM. Cell lysis and initial purification by phenolcellulose column-chromatography were carried out as described previously for purification of the wild-type enzyme (44). The pooled peak fractions from each preparation was further purified by the use of a 1 M HiTrap-heparin column (Amersham Biosciences, Inc.). The peak fractions were collected and were flash frozen in liquid nitrogen for storage at 4 °C. Purification of the active site tyrosine mutant DNA topoisomerase was examined by SDS-polyacrylamide gel electrophoresis, and protein concentrations of the fractions were estimated from spectrophotometric readings in the presence of Coomassie Blue (Pierce) using bovine serum albumin as a standard.

**RESULTS**

Growth of AS17 topA Cells in Media of Different Osmolarity—Fig. 1 depicts the plating efficiency of E. coli strain AS17 topA17/am pLL1(supD4347) cells on agar plates containing several thousand-fold in a low salt medium to about
105-fold in a high salt medium, was observed when the temperature was increased from 30 to 42 °C. Essentially the same results were obtained when sucrose instead of salt was added to the media to cover a similar range of osmolarity (data not shown). When the cells were transformed with pJW249 carrying a wild-type topA gene (46), the plating efficiency was no longer ts at any osmolarity, confirming that the observed changes reflected a topA phenotype.

The above results were surprising, however, when compared with similar data previously reported for strain BR83 topA17(am) supD43,74 (40). When the temperature was increased from 30 to 42 °C, the plating efficiency of BR83 cells was shown to decrease sharply in media of high osmolarity, but remain unchanged in broth containing no added osmolyte (40). Because both AS17 and BR83 were supposed to express an amber suppressor, the temperature-sensitive phenotype of AS17 cells was amplified by PCR and cloned into a single-copy plasmid pBeloBAC11. In addition, a lac promoter-linked topA cassette with a Y319A mutation (49), in which the active-site tyrosyl residue had been replaced by an alanine, was also moved into pBeloBAC11 to provide a topA null control in some of the experiments.

Complementation Assays of Mutant Proteins—The various constructs in the pBeloBAC11 vector were transformed individually into strain AS17 cells, and transformants picked from plates incubated at 30 °C were examined for growth at various temperatures. The results are tabulated in Table I. At a permissive temperature of 30 °C for strain AS17, all transformants were viable. At 42 °C, expression of wild-type topA or the mutant topA (G65N/W79S) fully complemented the inviability of AS17 cells; AS17 cells expressing topA(Y319A), topA(W79S), or the double mutant topA(G65N/W79S) showed a plating efficiency of less than 0.001, however. Thus in agreement with the postulate of a temperature-sensitive enzyme, the anticipated product of the topA17(am) allele in the presence of a functional supD suppressor is apparently inactive at 42 °C.

At an intermediate temperature of 37 °C, AS17 cells expressing topA(Y319A) showed a plating efficiency of less than 0.001, but the same cells expressing topA(W79S) and topA(G65N/W79S) showed significantly higher plating efficiencies of 0.14 and 0.04, respectively (Table I). Because the topA(G65N/W79S) double mutant appeared to be more stringent in its temperature sensitivity than the topA(W79S) single mutant, it was chosen for in vitro characterization of the mutant enzyme.

In Vitro Characterization of DNA Topoisomerase I with the G65N/W79S Substitutions—Wild-type E. coli DNA topoisomerase I and its mutated derivative TopA(G65N/W79S) were purified from strain DM800 ΔtopA cells harboring plasmid pJW312 or its mutated derivative. Both proteins were expressed to a comparable level upon induction of the lac promoter and were purified to apparently homogeneity.

Relaxation of negatively supercoiled plasmid DNA by the wild-type and mutant enzyme was first examined at 30 °C, in an assay mixture containing 20 mM Tris-HCl, pH 7.5, 100 mM...
KCl, 2.5 mM MgCl₂, 0.1 mM EDTA, and 100 μg/ml bovine serum albumin. As shown in Fig. 2, both the wild-type and mutant DNA topoisomerase I were capable of relaxing the negatively supercoiled DNA, but the latter was much less active. In 20 μl of the assay mixture containing about 300 ng of DNA, the bulk of the input negatively supercoiled DNA was relaxed after 20 min in the presence of 30–60 ng of the wild-type enzyme (Fig. 2, upper panel). Under the same conditions, the presence of 50 ng of the mutant enzyme converted only a minor fraction of the input DNA to topoisomers that migrated with significantly reduced mobilities (Fig. 2, lower panel). Even at the highest concentration of the mutant enzyme used in this experiment, the topoisomer products retained a significant number of negative supercoils (see the patterns of topoisomers in the lower panel of Fig. 2).

In Fig. 3, results of additional assays carried out in a buffer containing varying concentrations of KCl are depicted. The reduced activity of the mutant enzyme at 30 °C was again evident. Although 7.5 times more of the mutant enzyme was used relative to the wild-type enzyme in the two sets of reaction mixtures, relaxation of the negatively supercoiled plasmid DNA was generally less complete in the case of the mutant enzyme (compare the corresponding lanes in the upper left and upper right panels shown in Fig. 3). A shift of the assay temperature from 30 to 42 °C further reduced the relaxation activity of TopA(G65N/W79S) relative to that of the wild-type enzyme (compare the upper and lower right panels in Fig. 3 for the mutant enzyme and the upper and lower left panels in Fig. 3 for the wild-type enzyme). It was clear, however, that the mutant enzyme retained some activity at 42 °C; relaxation of negatively supercoiled plasmid DNA by the mutant enzyme was readily detectable, especially in assay mixtures containing lower amounts of KCl (see Fig. 3, lower right panel).

Cleavage of single-stranded DNA by the wild-type and TopA(G65N/W79S) was also examined. A 388-base pair-long DNA fragment uniquely 32P-labeled at a 5'-end was denatured and used in this experiment. Cleavage of the denatured DNA by 100 ng of the mutant enzyme or 20 ng of the wild-type enzyme in assay mixtures containing different amounts of KCl was performed at 30 °C (Fig. 4A). The mutant and wild-type enzyme appeared to cleave the DNA strand with the same sequence specificity, as similar ladders of labeled cleavage products were observed. Even though 5 times more of the mutant than the wild-type enzyme was used in the two sets of reaction mixtures, less cleavage was observed in samples containing the mutant enzyme. The reduced DNA cleavage activity of TopA(G65N/W79S) relative to wild-type DNA topoisomerase I was especially conspicuous in a low salt reaction buffer containing 10 or 40 mM KCl (compare lanes 1 and 10 and 2 and 11 of Fig. 4A). When the temperature was increased from 30 to 42 °C, the majority of the wild-type enzyme cleavage products showed little change in their intensities, indicating a lack of temperature sensitivity of cleavage at these sites by the wild-type enzyme. Increase or decrease in intensities was noticed for a few cleavage products, however (compare lanes 1 and 2 of Fig. 4B). Because DNA cleavage by E. coli DNA topoisomerase I is sensitive to the secondary structure of the DNA strand (50), these variations were likely a reflection of temperature-dependent changes in the secondary structure of the denatured DNA. For the mutant enzyme, on the other hand, the intensities of all detectable cleavage products were reduced upon a shift of the temperature from 30 to 42 °C (compare lanes 3 and 4 of Fig. 4B), suggesting a significant reduction of the DNA cleavage activity at the higher temperature. These results are thus similar to those described earlier for the relaxation of negatively supercoiled DNA. In both tests, TopA(G65N/W79S) is less active than the wild-type enzyme by roughly a factor of five to 10 even at a permissive temperature of 30 °C, and an increase of temperature to 42 °C further reduces the relaxation as well as the DNA cleavage activity of the mutant enzyme.

DNA Topoisomerase I Activity in Cells Expressing Wild-type topA or topA(G65N/W79S)—To further test whether the results obtained with purified enzyme preparations reflect their activities in vivo, the degree of supercoiling of a test plasmid pBR322 by cells expressing wild-type DNA topoisomerase I or TopA(G65N/W79S) was examined at 30 and 42 °C. In this experiment, E. coli strain DM800 cells, which carry a topA deletion and a compensatory mutation gyrB225, were first transformed with pBR322 and then with a pBeloBac11 derivative capable of expressing the wild-type or mutant topA coding region from a lac promoter. The doubly transformed cells were first grown in culture flasks placed in a 30 °C gyratory shaker, and equal portions of each culture were divided into two sets, one for continued growth at 30 °C and the other for growth in a 42 °C shaker bath. Ten minutes after splitting the cultures and restarting cell growth, cells in both sets of cultures were rapidly lysed and plasmid DNA samples were recovered for two-dimensional gel electrophoresis. In the results depicted in Fig. 5, three pairs of samples from cells expressing wild-type topA, topA(G65N/W79S), and topA(Y319A) were analyzed. In each pair, the sample on the left was recovered from cultures kept at 30 °C, and the sample on the right from cells 10 min after the temperature shift to 42 °C. For pBR322 from unheated cells expressing the inactive Y319A mutant enzyme, the topoisomer distribution (see the left half of the rightmost panel in Fig. 5) was similar to that of the same plasmid isolated from DM800 ΔtopA cells grown at the same temperature (result not shown). The topoisomers of different linking numbers were resolved into an arc, with an intense cluster near the lower tip of the arc (left half of the rightmost panel in Fig. 5). In this type of two dimensional gel electrophoresis, topoisomers of progressively lower linking numbers are distributed counterclockwise along the arc (45). As shown previously, pBR322 topoisomers that migrated near the extreme counterclockwise tip of the arc are about twice as negatively supercoiled as the same plasmid isolated from topA+ cells (51). The high intensity near the top of the arc in each sample was not significant, as nicked DNA was not well separated from the apex of the arc of the covalently closed topoisomers in this experiment.
Whereas expression of topA(Y319A) did not significantly change the linking number distribution of pBR322 in DM800 ΔtopA cells at 30 °C, expression of wild-type DNA topoisomerase I in the same cells effected a large shift in topoisomerase distribution (compare the left half of the leftmost panel to that of the rightmost panel in Fig. 5), indicating a large increase in the average linking number or a large reduction in the average degree of negative supercoiling. Significantly, the distribution of pBR322 topoisomers in the sample isolated from DM800 cells expressing TopA(G65N/W79S) at 30 °C (left half of middle panel in Fig. 5) was intermediate between those of samples isolated from the same unheated cells expressing the wild-type and Y319A enzyme. This shows that at 30 °C, the G65N/W79S mutant enzyme is active but not as active as the wild-type enzyme in vivo.

Upon shifting the growth temperature from 30 to 42 °C, the arc of topoisomers isolated from cells expressing wild-type (the rightmost panel in Fig. 5) or Y319A (the leftmost panel in Fig. 5) showed a clockwise shift, indicating that the temperature increase caused a reduction in negative supercoiling of the plasmid. In contrast, pBR322 isolated from cells expressing the G65N/W79S mutant enzyme showed a counterclockwise shift for the same change in growth temperature (Fig. 5, middle panel). It is known that the average degree of negative supercoiling of a plasmid is dependent on the temperature of cell growth (reviewed in Refs. 25, 26). Because this dependence is related to adaptation to thermal stress and may involve DNA topoisomerase I, gyrase, and a number of other proteins (see the reviews cited), interpretation of differences in linking number distributions of plasmids isolated from cells grown at different temperatures is often difficult. It is significant, however, that among the samples examined only pBR322 isolated from DM800 ΔtopA cells expressing TopA(G65N/W79S) showed an increase in the degree of negative supercoiling upon an upshift of growth temperature; samples of the same plasmid isolated from the same cells expressing wild-type or inactive DNA topoisomerase I all showed a decrease in the degree of negative supercoiling for the same temperature shift. These results are consistent with the expectation that increasing the tempera-
ture from 30 to 42 °C would decrease the intracellular activity of TopA(G65N/W79S), leading to an increase in negative supercoiling of the plasmid. Thus the results of the experiment depicted in Fig. 5 are in agreement with the in vitro results: TopA(G65N/W79S) is not as active as wild-type DNA topoisomerase I at 30 °C and its activity is further reduced at 42 °C.

**DISCUSSION**

We show in this work that the viability of AS17 topA17(am) cells bearing a plasmid-borne supD43,74 amber-suppressor is severely compromised at 42 °C in media of low as well as high osmolarity (see Fig. 1), indicating that DNA topoisomerase I is indispensable for growth of E. coli cells in these media. Previous studies of strain BR83 topA57(am) supD43,74 showed, however, that the cells were fully viable at 42 °C when grown in media of low osmolarity (40). Because neither strain showed temperature sensitivity in growth at any osmolarity upon transformation with a plasmid-born wild-type topA gene, the apparent contradiction summarized above could not be attributed to strain differences that are unrelated to the expression of a functional DNA topoisomerase I. We were therefore led to question the presumed common molecular basis of the temperature sensitivity of the two strains, namely the temperature-dependent suppression of a topA(am) mutation by supD43,74.

The possibility that the temperature sensitivity of strain AS17 might result from the synthesis of a temperature-sensitive mutant DNA topoisomerase I rather than the temperature-dependent suppression of an amber mutation was raised by the finding that the temperature sensitivity of BR83 cells in growth media of any osmolarity was lost by the introduction of the plasmid-borne supD from AS17 into these cells. This result suggested that the supD43,74 in BR83 was ts, but the same gene in strain AS17 had been altered and was no longer ts; thus introducing a temperature-independent suppressor into BR83 would abolish its temperature sensitivity. It then follows that the product of the topA(am) allele in the strain AS17 must itself be ts in order to explain the ts phenotype of the strain in the absence of a ts amber-suppressor.

The temperature sensitivity of the expected product of the strain AS17 topA allele was substantiated by several experiments. Expression of TopA(G65N/W79S), the expected product of topA17(am) in the presence of a functional supD, did not restore the viability of strain AS17 at 42 °C. Furthermore, relative to the wild-type enzyme, purified TopA(G65N/W79S) showed a reduced activity in the relaxation of negatively supercoiled DNA and cleavage of single-stranded DNA, even at 30 °C; when assayed at a higher temperature of 42 °C, the activity of the mutant enzyme was further reduced. Analyses of the linking number distributions of a test plasmid isolated from cells expressing wild-type DNA topoisomerase I, TopA(G65N/W79S), or TopA(V319A) also indicated that the in vitro DNA relaxation activity of TopA(G65N/W79S) mirrored its activity in vivo. TopA(G65N/W79S) showed a lower intracellular DNA relaxation activity than the wild-type enzyme at 30 °C, and its intracellular DNA relaxation activity appeared to be further reduced upon a shift of the growth temperature to 42 °C (Fig. 5).

Taken together, these in vitro and in vivo results provide strong evidence that the temperature sensitivity of strain AS17 is not tied to the temperature-dependent suppression of an amber mutation, but is the result of the supD-mediated synthesis of a mutant DNA topoisomerase I that is itself ts. The demonstration of a difference in the molecular basis of temperature sensitivity of strains AS17 and BR83 also provides an explanation of the apparent discrepancy that BR83 but not AS17 is viable in media of low osmolarity. It is most likely that a functional DNA topoisomerase I is required for growth of E. coli in media of any osmolarity, but the temperature sensitivity of the unaltered supD43,74 in strain BR83 may itself depend on the osmolarity of the growth medium; a lack of temperature sensitivity of supD43,74 at low osmolarity would account for the viability of BR83 cells grown at 42 °C in low osmolarity media (40).

The original supD43,74 allele on pLL1, which was used to transform strain AS17, was probably altered during the construction of the strain. All pLL1 preparations isolated from AS17 cells maintained in different laboratories gave no temperature-sensitive transformants of BR83 cells. It is plausible that even at 30 °C, an unaltered supD43,74 might be suboptimal for the production of sufficient amounts of TopA(G65N/W79S) in strain AS17 cells, resulting in a selective pressure for reversion or other alterations in the suppressor.

Our results also indicate that the mutation G65N by itself has rather limited effect on topA. Expression of a plasmid-borne topA(G65N) in strain AS17 cells was sufficient to restore growth at 42 °C. The mutation W79S, on the other hand, significantly reduces topA function at 42 °C, and its temperature sensitivity appeared to be further enhanced in combination with the mutation G65N (see Table I). In the crystal structure of a large fragment of E. coli DNA topoisomerase I, both Gly-65 and Trp-79 are located on a long loop that projects from a Rossman-like-fold. The locations of these amino acid residues are distant to the catalytic pocket for DNA breakage and rejoining (8). Thus the reduced activity of TopA(G65N/W79S) at 42 °C, and to a lesser extent at 30 °C as well, is unlikely to reflect a direct effect of the mutations on the catalysis of DNA breakage and rejoining by the enzyme. More likely, the W79S mutation in particular may affect the binding of the enzyme to DNA. The long loop extending from the Rossman-like-fold forms one bank of a groove, which, from crystallographic studies of E. coli DNA topoisomerases I and III, has been implicated in the binding of single-stranded DNA (8, 9, 52).

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