The Additional 165 Amino Acids in the B Protein of *Escherichia coli* DNA Gyrase Have an Important Role in DNA Binding*

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DNA gyrase is the only enzyme known to negatively supercoil DNA. The enzyme is a heterotetramer of A_2B_2 subunit composition. Alignment of the primary sequence of gyrase B (GyrB) from various species shows that they can be grouped into two classes. The GyrB of Gram-negative eubacteria has a stretch of about 165 amino acids in the C-terminal half, which is lacking in other GyrB subunits and type II topoisomerases. In *Escherichia coli*, no function has so far been attributed to this stretch. In this study, we have tried to assess the function of this region both *in vivo* and *in vitro*. A deletant (GyrBΔ160) lacking this region is non-functional *in vivo*. The holoenzyme reconstituted from gyrase A (GyrA) and GyrBΔ160 shows reduced but detectable supercoiling and quinolone-induced cleavage activity *in vitro*. GyrBΔ160 retains its ability to bind to GyrA and novobiocin. However, when reconstituted with GyrA, the deletant shows greatly impaired DNA binding. The intrinsic ATPase activity of the GyrBΔ160 is comparable to that of wild type GyrB, but this activity is not stimulated by DNA. These studies indicate that the additional stretch present in GyrB is essential for the DNA binding ability of *E. coli* gyrase.

Topoisomerases are a ubiquitous class of enzymes that catalyze the interconversion between different topological isomers of DNA (1, 2). Among these enzymes, DNA gyrase is the only enzyme that has the ability to negatively supercoil DNA (3, 4). In addition to supercoiling, gyrase can relax both positively and negatively supercoiled DNA and catalyze catenation/decatenation as well as knotting/unknotting reactions (4). Gyrase performs these topological transformations by creating transient double-stranded breaks in DNA and resealing the break after passing another DNA duplex through it (2). Hence, mechanistically the enzyme is a type II topoisomerase.

DNA gyrase is an essential enzyme and belongs exclusively to the prokaryotic kingdom. Although gyrases from various species show resemblance to eukaryotic type II topoisomerases, they have diverged significantly during evolution and have enzymatic properties distinct from other members of this class. Therefore, the enzyme is an effective target for many antibacterial agents (5, 6), some of which are used clinically as drugs. Two major classes of drugs that inhibit gyrase are quinolones and coumarins.

DNA gyrase is a heterotetramer consisting of two GyrA[1] and two GyrB polypeptides (4). The *gyrA* and *gyrB* genes have been cloned and sequenced from a variety of bacterial species (7), but most of the biochemical as well as the structural studies have centered on the enzyme from *Escherichia coli*. The GyrA and GyrB proteins have been shown to be organized as functional domains (4). The GyrA protein consists of an N-terminal domain (59–64 kDa) that harbors the DNA cleavage-reunion activity (8, 9) and a C-terminal 33-kDa region involved in wrapping the DNA (10). The GyrB protein consists of an N-terminal domain (43 kDa) that hydrolyzes ATP and binds to coumarin drugs (11, 12). The intrinsic ATPase activity of GyrB is stimulated by the presence of GyrA and DNA (13, 14). The C-terminal 47-kDa fragment of GyrB can complement the intact GyrA to form a complex that retains the ability to relax supercoiled DNA in the absence of ATP (11, 15). Therefore this part of the protein has been postulated to bind to GyrA and DNA. The exact region(s) involved in these functions have yet to be characterized.

Sequence alignment of GyrB proteins from different organisms (Fig. 1) reveals the absence of a stretch of about 165 amino acids from Gram-positive bacteria and mycoplasma (7, 16, 17). This stretch is also missing from eukaryotic topoisomerase II enzymes as well as from the homologues of GyrB in topoisomerase IV from different species. The structural information about type II topoisomerases is derived from the structure of an internal fragment of yeast topoisomerase II (18), the N-terminal ATPase domain of *E. coli* GyrB (19), and the N-terminal two-thirds of the *E. coli* GyrA (20). However, none of the structures include the 165-amino acid region. Furthermore, this region has not been implicated in any specific function.

In the present study, we have tried to assess whether the additional amino acid stretch present in GyrB of *E. coli* is essential for the function of the enzyme. We have created a truncated GyrB by deleting 160 amino acids within the extra 165-amino acid region in the full-length protein. The deletant GyrB (GyrBΔ160) has been purified, and its enzyme activities have been compared with GyrB. We have determined the maximal dispensable region in this particular stretch of amino acids without changing the ability of the protein to rescue the temperature-sensitive strain of GyrB. We find that the region is essential for the DNA binding ability of the active gyrase tetramer. Deletion of this stretch impairs the activity of the protein both *in vivo* and *in vitro*.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—E. coli DH10B was used for all cloning experiments. The strain N4177 (*gyrB^{ts} cou^r*) was a kind gift

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[1] The abbreviations used are: GyrA, DNA gyrase A protein; GyrB, DNA gyrase B protein; GyrBΔ160, GyrB protein lacking 160 amino acids; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay.

FIG. 1. **Alignment of primary sequences of GyrB homologues from different organisms.** The alignment includes GyrB from Gram-negative eubacteria (*EcB, E. coli*; *PpB, Pseudomonas putida*), Gram-positive eubacteria (*BsB, Bacillus subtilis*; *MtB, Mycobacterium tuberculosis*), archaea (*AfB, Archaeoglobus fulgidus*; *HaB, Haloferax alicantei*), and *Mycoplasma pneumoniae* (*MpB*); ParE from *E. coli* (*EcE*), GrlB from *Staphylococcus aureus* (*SaE*); and partial sequence of topoisomerase II from *Saccharomyces cerevisiae* (*Sct*) and *Homo sapiens* (*Hst*). The length of each sequence is shown on the *right*. Alignment was performed using MACAW software (35). *Blocks* denote regions of extended homology. Regions with greater than 67% similarity are in *black* and those with 37–67% similarity are in *gray*.
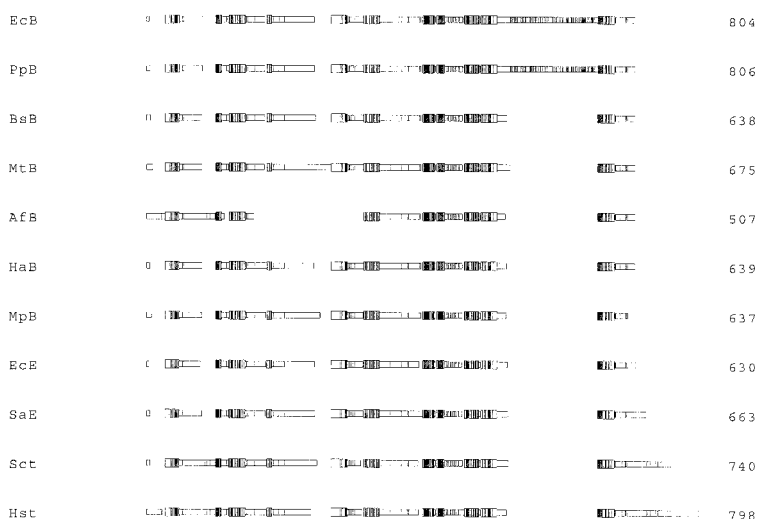
from M. Gellert (21). Overexpression plasmids pPH3 and pAG111 (22) were used for GyrA and GyrB purification, respectively. The plasmid pJW312-*Sal*I used in the purification of *E. coli* topoisomerase I was obtained from J. C. Wang (23).

DNA Manipulations—GyrBΔ160 was generated by PCR amplifying parts of the gene upstream and downstream of the 160-amino acid stretch using the primers P1 and P2 and primers P3 and P4, respectively. The primer sequences are as follows: P1, 5′-ACAGCGATGTC-GAATTCTTATG; P2, 5′-TTGCGCCCTCGAGCGCGATAGAGATC; P3, 5′-GTAGCCAGCCTCGAGCAGGCGCT; P4, 5′-GGCCAGTGCCAAGC-TTGCATG. The PCR products were digested with appropriate restriction enzymes and cloned (pMCN1 and pMCN2) separately in pBluescript KS(+) (Stratagene). The relevant portions were sequenced, and then the fragments were brought together in pBluescript KS(+) (pMCN3) resulting in the generation of an *Xho*I site at the junction. The *Sal*I-*Hin*dIII region of GyrB present in pAG111 was replaced with the *Sal*I-*Hin*dIII fragment from pMCN3, which encompasses the deletion to generate GyrBΔ160 (*pMCN4*, Fig. 2).

Two more deletions mutant of GyrB were created, one lacking the first 78 amino acids of the 160-amino acid stretch and the other where the following 84 amino acids were removed (*pMCN6* and *pMCN5*, Fig. 2). pMCN5 and pMCN6 were generated by replacing appropriate (*Sal*I-*Xho*I and *Xho*I-*Hin*dIII, respectively) fragments in pMCN4 with fragments from pAG111-*Xho*I.

For the purpose of generating a range of exonuclease III-mediated deletions (within the 160-amino acid stretch), an *Xho*I site was engineered near the center of the region in the full-length GyrB (pAG111-*Xho*I) (Fig. 2) using the QuikChange™ mutagenesis method (Stratagene). The mutagenesis was carried out using the following primers: 5′-GTCAGCGAACTCGAGGACAAAGAACAG and 5′-CTGTTCTTT-GTCCTCGAGTTCGCTGAC with pAG111 as the template. pAG111-*Xho*I linearized with *Xho*I was used as the substrate for the exonuclease III reaction (24). Aliquots were taken out at different time intervals (15–120 s), and the DNA ends were blunted using S1 nuclease and Klenow fragment of DNA polymerase I. After ligation, the DNA was transformed into strain N4177 for complementation studies.

Complementation Studies—gyrB*ts* strain N4177 was used for all complementation studies. After transformation, the cells were recovered at 30 °C and plated at the permissive (30 °C) or restrictive temperature (42 °C). In all the complementation experiments, cells transformed with pAG111 were used as a positive control and pTTQ18 as the negative control.

Enzymes and Substrate Preparation—GyrA and GyrB were purified as described previously (25). GyrBΔ160 was purified using the similar purification scheme as used for GyrB except that a gradient of 0–1 M NaCl was used to elute the protein from the Hi-Trap heparin-Sepharose column. GyrBΔ160 was also purified using a novobiocin-Sepharose affinity column as described previously (25). The protein was purified from N4177 cells harboring pMCN4. Supercoiled pUC18 and pBR322 were prepared by standard DNA purification protocols (24). *E. coli* topoisomerase I was purified, and relaxed pUC18 was prepared as described by Lynn and Wang (23).

Assays—Supercoiling assays were carried out as described by Mizzuuchi *et al.* (26). Ciprofloxacin-induced cleavage was performed in supercoiling buffer except that ATP was omitted and supercoiled
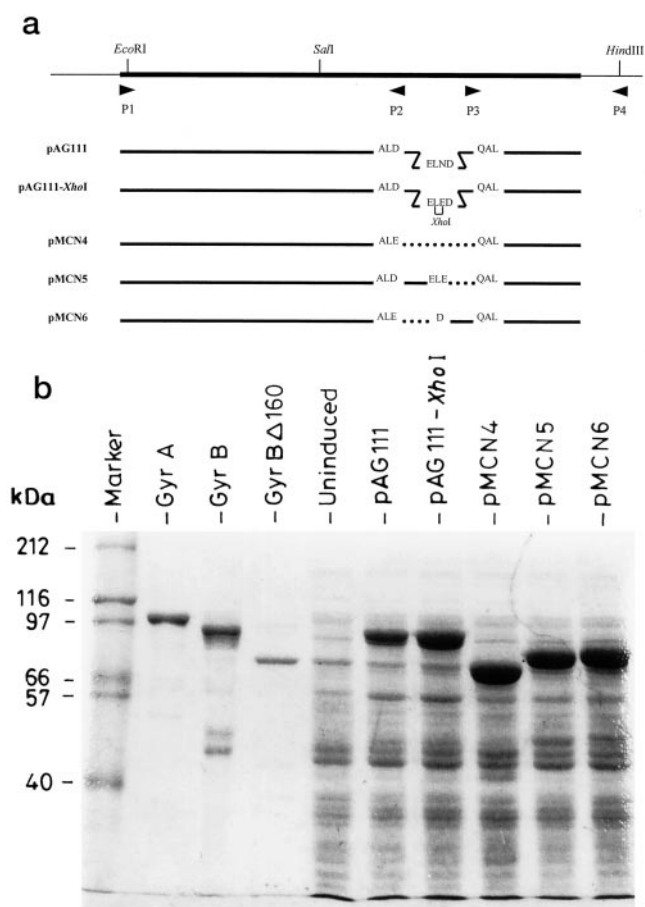

FIG. 2. **Derivatives of GyrB used in this study.** *a,* schematic representation of various GyrB derivatives. The relevant restriction sites have been denoted on the *gyrB* gene (*thick line*). Derivatives of the GyrB protein are shown with the junctions generated as a result of various deletions. The *Xho*I site engineered in pAG111-*Xho*I causes a single amino acid change (N637E). The regions between Ala-559 and Gln-720, Glu-635 and Gln-720, and Ala-559 and Asp-638 are deleted in pMCN4 (GyrBΔ160), pMCN5, and pMCN6, respectively. In all three deletants, two amino acids (lysine and glutamate) are added at the junction. *b,* induction profile of N4177 cells transformed with different constructs. The cells were grown at 30 °C until A_{600} = 0.5 and induced with 50 µM isopropylthiogalactoside for 4 h. Purified GyrA, GyrB, and GyrBΔ160 are also shown.

pBR322 was used as the substrate. Ciprofloxacin was added at a final concentration of 100 µg/ml. The reaction was carried out at 37 °C for 30 min and the drug-gyrase-DNA complex was trapped by adding 0.2%
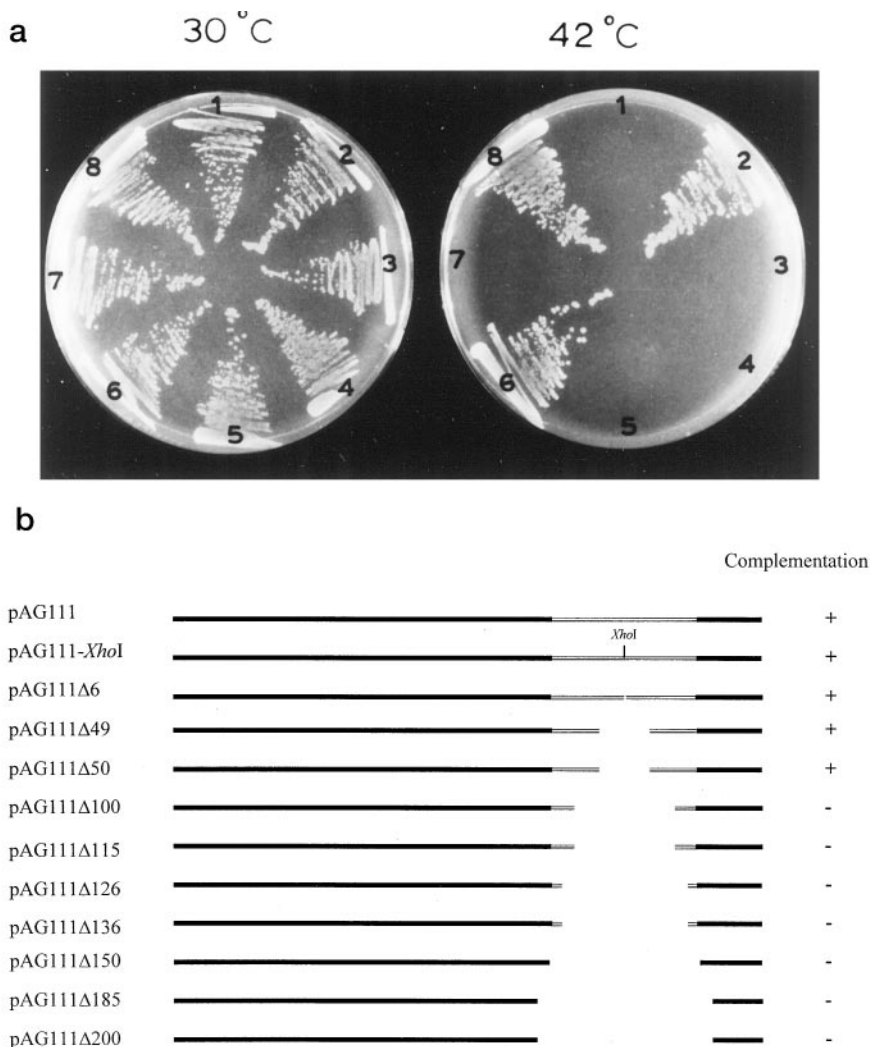
FIG. 3. ***In vivo* complementation analysis of *gyrB^{ts}* strain by various deletants.** *a*, N4177 (*gyrB^{ts}*) cells were transformed with pTTQ18 (*region 1*), pAG111 (*region 2*), pMCN4 (*region 3*), pMCN5 (*region 4*), pMCN6 (*region 5*), and pAG111-*Xho*I (*region 6*). Cells transformed with representative non-complementing (*region 7*) or complementing (*region 8*) clones generated in the exonuclease III experiment are also shown. *b*, schematic representation of exonuclease III-mediated deletions. All clones overexpress the protein of the expected size.

SDS. After 5 min, proteinase K was added at a final concentration of 0.8 mg/ml and incubated for 30 min. The reaction mixtures were resolved on 0.8% agarose gel in 40 mM Tris acetate buffer containing 1 mM EDTA. The gel was run at 1.5 V/cm for 6 h.

ATPase Assays—ATPase assays were performed as described previously (27). The reactions (30 μl each) were carried out in supercoiling buffer containing 2 mM ATP and 0.04 μCi of [α-^{32}P]ATP (>5000 Ci/mmol, NEN Life Science Products). The reaction was performed at 37 °C for 30 min and terminated by adding chloroform. 5 μl of the aqueous layer was resolved on a polyethyleneimine-cellulose thin layer chromatography plate (Merck), which was developed with 1.2 mM LiCl and 0.1 mM EDTA. The spots corresponding to ADP and ATP were quantitated using a PhosphorImager (Fuji Film FLA2000). Sonicated salmon sperm DNA (150 μg/ml) was used wherever indicated.

Electrophoretic Mobility Shift Assay—EMSA was performed using a PCR-amplified 240-base pair fragment encompassing the preferred gyrase cleavage site in pBR322 (28). The primers used for the amplification were end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase prior to PCR. The labeled DNA fragment was gel-eluted and purified through Sephadex G-50 (Amersham Pharmacia Biotech). Gyrase was reconstituted in supercoiling buffer (tRNA and ATP were omitted) and incubated with labeled DNA at 4 °C for 1 h. The free DNA and the gyrase-DNA complex were separated using 5% polyacrylamide gel (29:1 acrylamide/*N,N*′-methylenebisacrylamide) in 90 mM Tris borate, 5 mM MgCl$_2$. The gel was run at 4 °C at 5 V/cm for 8 h, autoradiographed, and quantitated using a PhosphorImager.

Novobiocin-Sepharose Column—Novobiocin was coupled to Sepharose as described before (25). GyrB was loaded, and the column was washed with TGED (TGED, 50 mM Tris-HCl (pH 7.5), 5% (v/v) glycerol, 1 mM EDTA, and 1 mM dithiothreitol). GyrA was loaded, and the column was successively washed with TGED containing 0 M KCl, 1 M KCl, 2 M urea, and 6 M urea. Samples from each wash were analyzed by SDS-

polyacrylamide gel electrophoresis to determine the elution of GyrB and GyrA. GyrBΔ160 was also analyzed under similar experimental conditions.

Surface Plasmon Resonance—Surface plasmon resonance experiments were performed on a BIAcore 2000 system (BIAcore). GyrA was immobilized on the CM5 sensor chip via amine coupling in acetate buffer (pH 4.5). The surface was blocked with ethanolamine hydrochloride. The interaction was assessed in 10 mM HEPES-NaOH (pH 7) containing 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol. Proteins used for the experiment were dialyzed against running buffer prior to the experiment. Different concentrations of GyrB and GyrBΔ160 were passed over the immobilized GyrA, and the subsequent changes in resonance units were recorded. Buffer containing 1 M KCl was used to dissociate bound GyrB or GyrBΔ160 from GyrA after each run.

RESULTS

Alignment of the primary sequences of type II topoisomerases shows the presence of an extra 165-amino acid stretch in GyrBs of Gram-negative organisms (Fig. 1). ParE, the GyrB homologue in topoisomerase IV from *E. coli*, however, lacks this region. GyrB from archaea as well as topoisomerase II from eukaryotes also do not have this region. To assess the function of this stretch, we created a deletant of *E. coli* GyrB that lacked 160 amino acids from the C-terminal domain of the protein (*pMCN4,* Fig. 2*a*). In addition we also generated two "partial" deletants that lack either the N-terminal (pMCN6) or the C-terminal (pMCN5) halves of the 160 amino acids.

GyrB Lacking 160 Amino Acids Fails to Rescue the gyrB^{ts} Strain—The *E. coli* strain N4177 carries a temperature-sensi-

tive allele of *gyrB*. pAG111 (plasmid expressing full-length GyrB) is able to rescue the cells at the restrictive temperature. On the other hand, cells expressing any of the three deletants were unable to grow at restrictive temperature (Fig. 3*a*) even when the expression was induced with isopropylthiogalactoside. Comparable amounts of the deletant proteins were present in cells with respect to the expression of full-length GyrB (Fig. 2*b*), and around 90% of the proteins were soluble after induction (data not shown). Furthermore, immunoblots using monoclonal antibody against GyrB showed that the steady state level of the deletants was very similar to GyrB in the uninduced state. Thus the deletants were being expressed and had similar stability *in vivo* as the full-length GyrB. These results imply that the inability to rescue the *gyrB*[ts] strain was due to the impaired activity of the deletants *in vivo* and not because of their lack of expression, solubility, or rapid degradation.

Exonuclease III-mediated Deletions—A range of deletions within the 165-amino acid region was generated using exonuclease III. Restriction analysis and the protein expression profile indicated that a large range of in-frame deletions was obtained. Under our experimental conditions, no clones were obtained that had the entire 165-amino acid region removed and yet was able to rescue *gyrB*[ts] strain (Fig. 3*b*). Among the clones that rescued the *gyrB*[ts] strain, those harboring the largest deletions were sequenced to determine the precise junctions. The largest region that could be removed without hampering the protein function *in vivo* was comprised of the central 50 amino acids (621–670, Fig. 3*a, segment 8*).

The Deletant Shows Reduced Supercoiling and Cleavage Activity in Vitro—The GyrBΔ160 protein was purified from N4177 cells harboring pMCN4 (see "Experimental Procedures," Fig. 2*b*). The ability of the purified GyrBΔ160 to support supercoiling in the presence of GyrA and ATP was assessed at 30 and 42 °C. The protein showed extremely reduced (<2% of the wild type) supercoiling activity at both temperatures. Fig. 4*a* depicts one such supercoiling assay performed at 42 °C. In addition, the activity was inhibited in the presence of novobiocin. It is noteworthy that the strain N4177 harbors a temperature-sensitive allele of *gyrB*, which shows no detectable activity at 42 °C (21, 29) and is resistant to novobiocin at 30 °C (21). Thus, the above results clearly demonstrate that the supercoiling activity is specific to the holoenzyme composed of GyrA and GyrBΔ160. Quinolones (*e.g.* ciprofloxacin) are known to trap the covalent enzyme-DNA intermediate in the topoisomerization reaction and have been used to assess the cleavage activity of DNA gyrase holoenzyme (30). The deletant showed 80-fold reduction in ciprofloxacin-induced cleavage, paralleling the results obtained with the supercoiling assay (Fig. 4*b*).

Deletant Binds to Novobiocin and GyrA—To ensure that the reduced specific activity was not due to improper folding of the deletant, the other properties exhibited by GyrB were examined. The N-terminal region of GyrB is known to bind to coumarins (*e.g.* novobiocin). GyrB interacts very tightly to novobiocin, and this has proved useful in the purification of the protein (13). Novobiocin coupled to epoxy-activated Sepharose serves as an affinity handle to assess the binding of gyrB to the drug. Moreover, GyrB bound to novobiocin on the column retains its ability to interact with GyrA. The deletant was retained, when passed through such a novobiocin-affinity column, exhibiting the property characteristic of wild type GyrB. In addition, GyrA interacted with the deletant bound to the column (not shown) indicating that the overall tertiary structure of the deletant was unaltered. The GyrBΔ160 and GyrA interaction was quantitatively evaluated using the BIAcore system. For this purpose, GyrA was immobilized on a CM-5
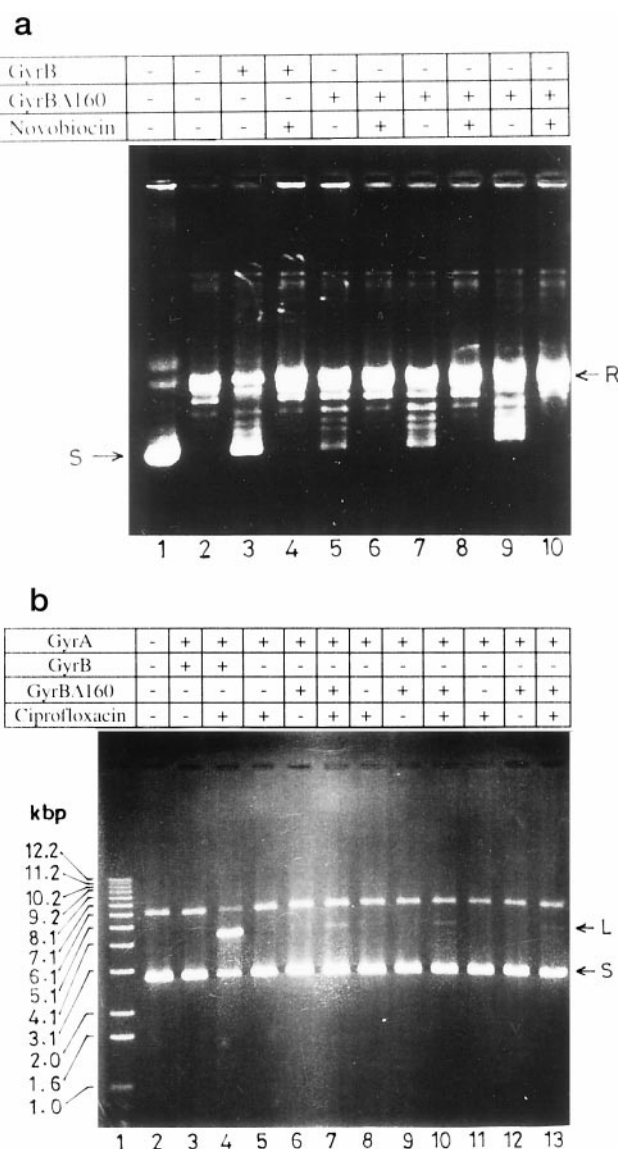

FIG. 4. **Holoenzyme comprising GyrA and GyrBΔ160 shows reduced supercoiling and cleavage activity.** *a*, supercoiling activity, 30 pmol of GyrA was used in all reactions. 0.3 pmol of GyrB was added in *lanes 3* and *4*. GyrBΔ160 was added as follows: 1.5 (*lanes 5 and 6*), 5 (*lanes 7 and 8*), and 15 pmol (*lanes 9 and 10*). Novobiocin (60 pmol) was added wherever indicated. The reactions were performed at 42 °C for 30 min. *Lanes 1* and *2* have supercoiled (*S*) and relaxed (*R*) pUC18, respectively. *b*, ciprofloxacin-induced cleavage reaction; GyrA was added as follows: 6 (*lanes 3 and 4*), 30 (*lanes 5–7*), 10 (*lanes 8–10*), and 3 pmol (*lanes 11-13*). 3 pmol of GyrB was added in *lanes 3* and *4*. GyrBΔ160 was added as follows: 15 (*lanes 6 and 7*), 5 (*lanes 9 and 10*), and 1.5 pmol (*lanes 12 and 13*). Ciprofloxacin (100 µg/ml) was added wherever indicated. The covalent gyrase-DNA complex was trapped with SDS (0.2%) and digested with proteinase K (0.8 mg/ml) for 30 min at 37 °C. Supercoiled pBR322 (*S*) was used as the substrate for the reaction, and the formation of linear pBR322 (*L*) was monitored. *Lane 1* shows 1-kilobase pair ladder (Life Technologies, Inc.).

sensor chip. Various concentrations of analyte (GyrB or GyrBΔ160) were passed over the sensor surface, and the subsequent changes in resonance units were recorded. The kinetic parameters obtained show that the deletant bound to GyrA with an affinity comparable to the full-length GyrB (Table I). Furthermore, when GyrB was immobilized and GyrA was passed over the surface, similar results ($K_D = 2.1 \times 10^{-7}$ M) were obtained, substantiating the authenticity of the interaction.

The Holoenzyme Comprising GyrA and GyrBΔ160 Is Impaired in DNA Binding—EMSAs were carried out to assess the

TABLE I
Kinetic parameters of the interaction of GyrA with GyrB and GyrBΔ160

Protein	k_{on}	k_{off}	K_D
	$M^{-1} s^{-1}$	s^{-1}	M
GyrB	$1.73 \times 10^3 \pm 0.21 \times 10^3$	$1.17 \times 10^{-3} \pm 0.12 \times 10^{-3}$	$6.83 \times 10^{-7} \pm 1.63 \times 10^{-7}$
GyrΔ160	$0.93 \times 10^3 \pm 0.19 \times 10^3$	$0.32 \times 10^{-3} \pm 0.07 \times 10^{-3}$	$3.47 \times 10^{-7} \pm 0.21 \times 10^{-7}$

FIG. 5. **Holoenzyme comprised of GyrBΔ160 fails to bind to DNA.** EMSAs were performed with holoenzyme composed of GyrA-GyrB (*a*) and GyrA-GyrBΔ160 (*b*). 300 fmol of DNA and 200 nM GyrA were used in all reactions. Varying amounts of GyrB (*a*) and GyrBΔ160 (*b*) were added as indicated. The reactions were incubated for 1 h at 4 °C and resolved in a 5% polyacrylamide gel.
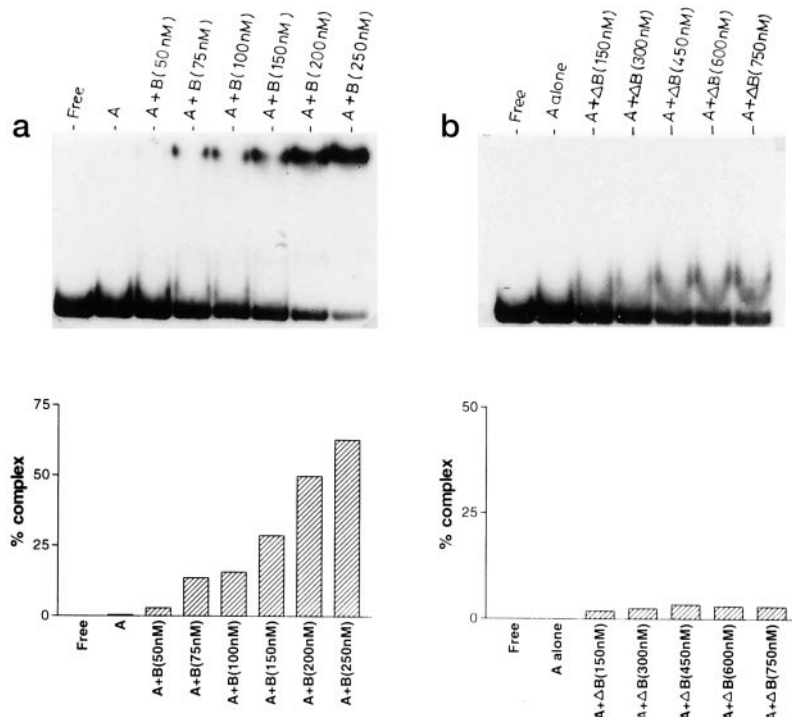
binding of gyrase to DNA. The holoenzyme comprising GyrA and GyrB bound to DNA in a concentration-dependent manner (Fig. 5*a*). However, the enzyme reconstituted from GyrA and GyrBΔ160 showed no detectable stable complex even when much higher amounts of protein were used (Fig. 5*b*). Thus, it appears that the removal of the 165-amino acid region from GyrB affects the DNA binding ability of the holoenzyme. Since the deletant binds to GyrA, we wanted to assess its effect on the formation of A$_2$B$_2$ holoenzyme in an EMSA reaction. When GyrA was mixed with fixed and limiting amount of GyrB and varying amounts of GyrBΔ160, there was a concentration-dependent increase in the amount of complex formed (Fig. 6*a*). However, with limiting GyrA, there was reduction in the amount of gyrase-DNA complex formed with the increase in GyrBΔ160 (Fig. 6*b*). These results are in accordance with a scenario where GyrBΔ160 is defective in DNA binding but not in its interaction with GyrA (see "Discussion").

The ATPase Activity of the Deletant Is Not Stimulated by the Presence of GyrA and DNA—The ATPase activity of GyrB and GyrBΔ160 was assessed in the absence and presence of DNA. The intrinsic ATPase activity of the enzymes in the absence of DNA was similar (Fig. 7, K_m, GyrB, 1.4 mM and GyrBΔ160, 1.6 mM). In the presence of DNA, there was stimulation in ATP hydrolysis by GyrB, whereas the deletant showed no appreciable change in activity. Taken together, these findings agree well with the failure of the A$_2$ΔB$_2$ holoenzyme to bind DNA.

DISCUSSION

The present study is an attempt to understand the function of the extra 165 amino acids present in GyrB of *E. coli*. We find that the region plays an essential role in the functioning of *E.*

coli DNA gyrase. This region is present only in Gram-negative organisms. In contrast, other homologues of GyrB in all three kingdoms (eukarya, archaea, and Gram-positive eubacteria) lack this region. It is also absent from topoisomerase IV belonging to both Gram-positive and Gram-negative eubacteria.

Our analysis shows that truncated *E. coli* GyrBs, lacking the stretch (GyrBΔ160), or lacking half of the region are unable to rescue a *gyrB^ts* strain (Fig. 3). Thus the deletants appear to be non-functional *in vivo* despite being expressed at high levels with >90% in the soluble form. In addition, exonuclease III-mediated deletion analysis revealed that deletions greater than 50 amino acids (near the center of the stretch) rendered the protein inactive *in vivo*. This further confirms that the stretch is essential for the functioning of GyrB in *E. coli*.

We purified GyrBΔ160 to analyze its biochemical properties *in vitro*. The purified GyrBΔ160 retains novobiocin binding (not shown), GyrA binding (Table I), and has similar intrinsic ATPase activity to full-length GyrB (Fig. 7*a*). Thus, it appears that the removal of 160 amino acids within the 165-amino acid stretch does not grossly disturb the overall conformation of the protein. In contrast, GyrBΔ160 (in presence of GyrA) is severely compromised in both supercoiling activity and drug-induced cleavage activity (Fig. 4). Therefore, it appears that the deletant is dysfunctional in cleavage or a step prior to it. In the supercoiling reaction cycle of DNA gyrase, prior to cleaving DNA, GyrB has to bind to GyrA and form an active heterotetramer. Thus, the reduced cleavage by the deletant may be due to its inability to bind GyrA, inefficient heterotetramer formation, reduced DNA binding ability, or poor cleavage activity *per se*. We find that the deletion does not affect the interaction of
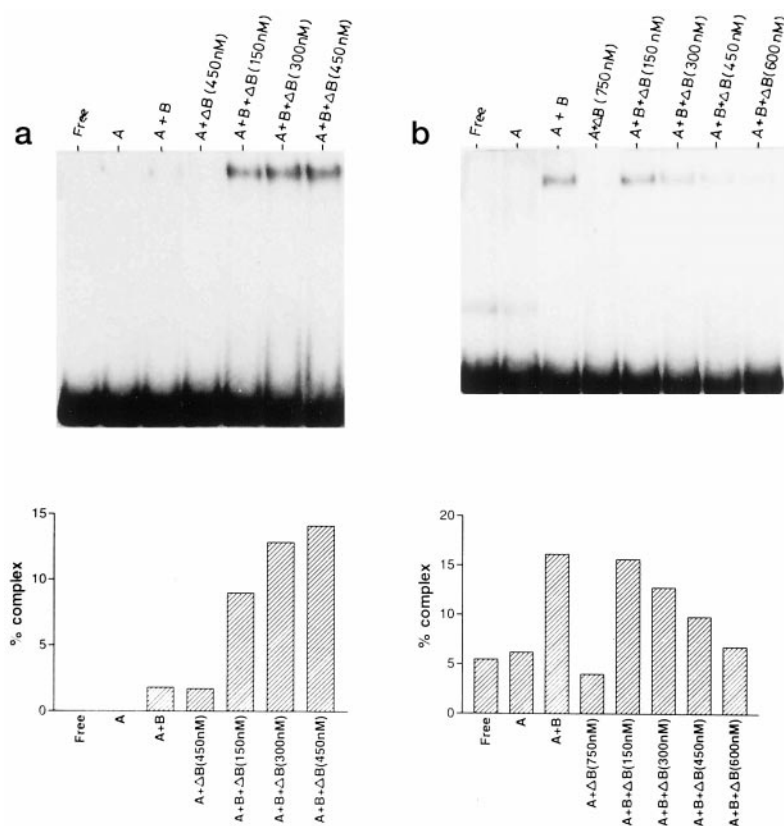
FIG. 6. **The presence of GyrBΔ160 alters the complex formation by wild type gyrase.** *a*, EMSA in the presence of excess GyrA, 300 fmol of DNA, and 200 nM GyrA was used in all reactions. 50 nM of GyrB was used wherever indicated. *b*, EMSA in the presence of limiting GyrA, 30 fmol of DNA, and 30 nM GyrA was used in all reactions. 150 nM GyrB was used wherever indicated. Varying amounts of GyrBΔ160 were added as mentioned.

GyrB with GyrA (Table I); however, the deletant shows a drastic reduction in DNA binding. There is no detectable stable gyrase-DNA complex seen in EMSAs with the gyrase ($A_2\Delta B_2$) holoenzyme (Fig. 5*b*). Under similar conditions, the wild type (A_2B_2) holoenzyme is able to bind DNA and form a stable complex (Fig. 5*a*).

Previous work has suggested that GyrB readily forms heterodimers when two populations are mixed (31, 32). Therefore, it is possible to calculate the proportions of wild type homodimers, mutant homodimer, and heterodimer in a mixed population. This provides an explanation for the data shown in Fig. 6. In Fig. 6*a*, where the concentration of GyrA is high, increasing amounts of ΔB lead to an increase in the percentage of DNA-protein complex. If we assume that $A_2B\Delta B$ can bind DNA, then the increase in the amount of this complex will lead to an increase in the total amount of DNA bound. In Fig. 6*b*, where the concentration of GyrA is limiting, there will be a competition between the B dimers for GyrA. With increasing amounts of ΔB, $A_2\Delta B_2$ will predominate. As this species is known to be incapable of binding to DNA (Fig. 5), the amount of DNA bound will decrease, as observed. Therefore, these data support the idea that GyrB exists as a monomer-dimer equilibrium and suggest that the heterodimer ($A_2B\Delta B$) is capable of binding to DNA. Taken together, these results indicate that the deletant is able to bind GyrA but is defective in DNA binding. It has been shown earlier that the ATPase activity of GyrB is stimulated in the presence of GyrA and DNA (13, 14). The intrinsic ATPase activity of the deletant is similar to the wild type (Fig. 7*a*). However, this activity is not stimulated in the presence of GyrA and DNA, supporting the above conclusion.

Topoisomerization reaction, especially supercoiling, involves a series of complicated steps. In the case of gyrase, strand transfer requires a duplex to pass through the entire dimer interface of the protein. During this passage, one would expect a series of transient DNA-protein interactions. Thus, it is not surprising that more than one region in DNA gyrase partici-
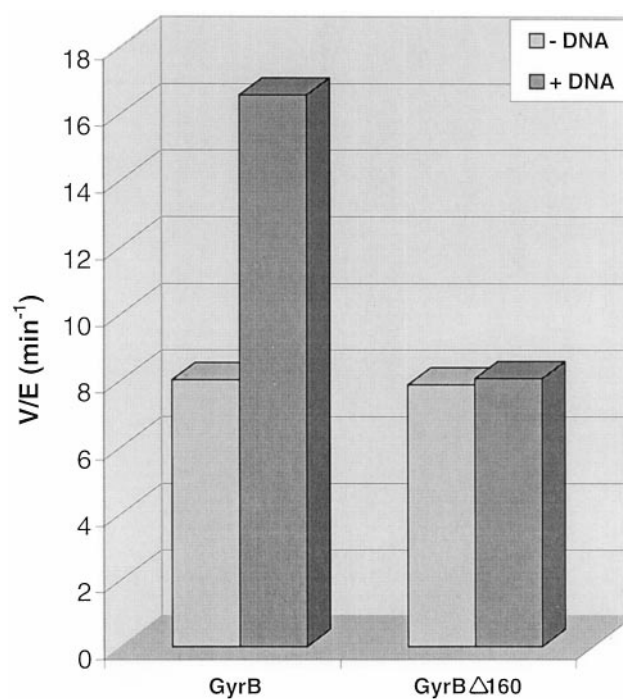

FIG. 7. **Kinetics of ATP hydrolysis.** Reactions were performed with 600 nM GyrB or GyrBΔ160. 800 nM GyrA and 1 mM ATP was present in all reactions. The +DNA reactions contained, in addition, 150 μg/ml sonicated salmon sperm DNA. The reactions were incubated at 37 °C for 30 min and terminated with chloroform. The samples were resolved on a polyethyleneimine cellulose thin layer chromatography plate.

pates in DNA binding. Full-length GyrA, as well as the C-terminal domain of GyrA alone, can bind to DNA in the absence of GyrB, albeit with low affinity (10). The C-terminal 33-kDa region of GyrA is known to wrap DNA in a positive superhelical sense. In addition, the N-terminal two-thirds of GyrA contains

the active site tyrosine (4) and is implicated in both covalent and non-covalent interactions with DNA (20). In the case of GyrB, there is indirect evidence for DNA binding from at least three different observations. First, the ATPase activity of GyrB is stimulated by the presence of DNA in the central cavity within the GyrB dimer (33). Second, GyrB not only enhances the stability of the protein-DNA complex but its presence is essential for the manifestation of the cleavage activity by GyrA. In accordance with this, the B′ region of yeast topoisomerase II (equivalent to the C-terminal half of GyrB) has been shown to harbor amino acids crucial for the cleavage-reunion activity of the enzyme (34). Third, the C-terminal 47 kDa of GyrB in the presence of GyrA can support ATP-independent relaxation (15). Therefore, it has been proposed to be involved in binding both DNA and GyrA. However, no precise region(s) in GyrB has been allocated these functions. Our findings indicate that the domain/region within the 165-amino acid stretch may, directly or indirectly, be involved in DNA binding.

The 165-amino acid region in GyrB is a characteristic of Gram-negative eubacteria. Our extensive sequence analysis has failed to reveal any significant homology with other proteins. Although the stretch shows moderate conservation among different species, we failed to detect any sequence motif, which would be indicative of its function. The occurrence of other functional gyrases lacking this stretch seems to imply that this region may be dispensable. On the contrary, we find that the 165-amino acid stretch is essential in *E. coli* GyrB both *in vivo* and *in vitro*. This raises the possibility that among gyrases there may be two classes of enzymes that show subtle differences in their interaction with DNA. A detailed comparative analysis of gyrase from Gram-positive and Gram-negative organisms needs to be done to understand better the functioning of these proteins and their evolutionary history. It would also be interesting to look for the types of compensatory mechanisms/mutations operating in gyrases lacking this stretch.

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