An Open Conformation of the Thermus thermophilus Gyrase B ATP-binding Domain*

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DNA gyrase forms an $A_2B_2$ tetramer involved in DNA replication, repair, recombination, and transcription in which the B subunit catalyzes ATP hydrolysis. The Thermus thermophilus and Escherichia coli gyrases are homologues and present the same catalytic activity. When compared with that of the E. coli 43K-5′-adenylyl-β,γ-imidodiphosphate complex, the crystal structure of Gyrase B 43K ATPase domain in complex with novobiocin, one of the most potent inhibitors of gyrase shows large conformational changes of the subdomains within the dimer. The stabilization of loop 98–118 closing the active site through dimeric contacts and interaction with domain 2 allows to observe novobiocin-protein interactions that could not be seen in the 24K-inhibitor complexes. Furthermore, this loop adopts a position which defines an “open” conformation of the active site in absence of ATP, in contrast with the “closed” conformation adopted upon ATP binding. All together, these results indicate how the subdomains may propagate conformational changes from the active site and provide crucial information for the design of more specific inhibitors.

Type II topoisomerases are enzymes essential for chromosome segregation and cell division due to their ability to modify the topological forms of procaryotic and eukaryotic DNA (1). The topoisomerases II share sequence, functional, and structural similarities and this knowledge comes mostly from complementary comparative studies done on the procaryotic and eukaryotic enzymes (2–5). At difference with the eukaryotic enzyme, the bacterial enzyme named gyrase, catalyzes the negative supercoiling of DNA and consists of two proteins, namely GyrB and GyrA associated into an $A_2B_2$ oligomer. ATP binding and hydrolysis in the N-terminal part of the B subunit appears to be required for protein-protein interactions and recycling of the enzyme (6). This part of the protein also contains the entry site for the DNA T-segment (7–9) and studies have shown that this domain behaves as an ATP-operated clamp which binds DNA during the supercoiling cycle (10).

Recent structural studies on yeast topoisomerase II have shown that the domains of this modular enzyme are capable of wide conformational changes which could be correlated with the topoisomerization mechanism (4). Nevertheless, the structure of the whole enzyme is not known. Up to now, the only structural information from the ATP-binding site comes from the Escherichia coli 43K complex with ADPPNP (6, 11). Most of the residues which bind the ATP molecule lie in the 24-kDa N-terminal region between residues 1 and 220 (domain 1), but there are two residues (Gln$^{335}$ and Lys$^{337}$) in domain 2 (residues 220–392) which also contact the ATP molecule. Although the role of these highly conserved residues remains unclear, mutational studies have pointed out their role in the hydrolysis of ATP and they are thought to be implicated in the transmission of conformational changes upon ATP hydrolysis (12, 13). The loop closing the active site and comprising residues 98 to 118 forms a β-sheet with the N-terminal arm of the other monomer containing essential residues for the dimerization process, among them is the conserved Ile$^{10}$ (6, 14). The loop 98–118 is implicated in the binding and hydrolysis of ATP and is composed of conserved residues among the gyrase family (15) which are involved in the catalytic mechanism (16–18).

The inhibition of this enzyme has been extensively studied because of the relative sequence divergence in the different pathological bacterial strains and between the prokaryotic and eukaryotic enzymes for the design of specific antibiotics or alternatively antitumoral drugs. It has been suggested that diverse inhibitors of type II eukaryotic and procaryotic topoisomerases such as quinolones and etoposides share a common binding site and a common mechanism of action (19). In each case, inhibition of the type II DNA topoisomerases yields a complex of enzyme covalently bound to cleaved DNA (20). Another group of molecules, namely the coumarins, were previously designed to target the ATP-binding site of gyrase B from bacterial pathogens (21–23).

The crystallographic structure of domain 1 (residues 1–220) of the gyrase B subunit from different strains, a 24-kDa protein, has been solved in complex with several coumarins, one of the most potent families of gyrase inhibitors (24, 25). Some residues in the pocket are specifically dedicated to the novobiocin binding and mutations at these positions induce the propagation of resistant bacterial strains. Nevertheless, it was shown that the 24 kDa is unable to bind and hydrolyze ATP nor to bind ADP or ADPPNP (26). No structural data are available on the conformation adopted by the whole 43K ATP-binding domain in the presence of a coumarin inhibitor.

We present here the first structural data available on the complex between the entire N-terminal domain of GyrB (43K) and novobiocin, one of the most potent inhibitors of gyrase. We used the 43K domain of gyrase B from the thermophilic bacteria Thermus thermophilus which presents a wider temperature range of activity than the mesophile E. coli bacteria. The particular properties of the thermophile enzyme allowed us to

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† The abbreviation used is: ADPPNP, 5′-adenylyl-β,γ-imidodiphosphate.
crystallize the N-terminal domain dimer in a new conformation. At the same time, this structure provides information on the ATP binding pocket conformational state targeted by novobiocin.

**EXPERIMENTAL PROCEDURES**

**In Vitro Studies on the T. thermophilus A2B2 Complex**—The details of the cloning of *T. thermophilus* Gyrase A and Gyrase B and of the determination of optimum temperature will be published elsewhere. Negative supercoiling of relaxed pBR322 plasmid was carried out as described by Brino et al. (14, 27). After 30 min of incubation at 25 °C, reactions were stopped by addition of 1% SDS-DNA gel loading buffer. The decatenation activity was determined using kinetoplast DNA reactions were stopped by addition of 1% SDS-DNA gel loading buffer.

**T. thermophilus 43K Domain Expression and Co-crystallization with Novobiocin**—The details of the cloning, purification, and crystallization of the *T. thermophilus* 43K domain of Gyrase B will be described elsewhere. Briefly, the *T. thermophilus* 43K domain from the initial Met to residue 392 was overexpressed in *E. coli* and purified using a three-step protocol ending with a size exclusion chromatography. The 43K protein was concentrated to 4.5 mg ml⁻¹ in a buffer containing 10 mM Tris, pH 7.2, 1 mM EDTA, pH 8.0, 5 mM 2-mercaptoethanol, and 200 mM NaCl. The initial screening using Crystal Screen 1 and 2 from Hampton Research was carried out with a protein/novobiocin ratio of 1:1.2 at 276 K and 295 K. We obtained two crystal forms, one with PEG8000 and the other with sodium formate as a precipitating agent. The crystals obtained using sodium formate at pH 8.5 appeared within 3 days at 295 K and could be directly frozen from the mother liquor using a cryoloop. Despite their size (∼10 × 80 × 60 μm³), the crystals diffract up to 2.3 Å on beamline ID14-EH4 (ESRF, Grenoble, France) and a full dataset could be collected with a single crystal. The crystals belong to the P2₁ space group with a = 44.9 Å, b = 125.5 Å, c = 79.8 Å, and β = 96.4° with 2 molecules per asymmetric unit.

**Structure Determination**—The data were processed using DENZO and SCALEPACK programs (28) between 15 and 2.3 Å (R_free = 6.4% with redundancy of 2.6 and completeness of 96.6%). The structure was solved by molecular replacement using one *E. coli* 43K monomer (61) (all residues replaced by alanines) as a search model with the program AmoRE (29). The molecular replacement calculations gave two solutions in the asymmetric unit with a correlation coefficient of 21.1% and a free R factor of 54.5% after fast rigid body refinement. The two molecules are related by a 2-fold noncrystallographic axis. Iterative cycles of rigid body refinement and torsion angle molecular dynamics at 4000 K in crystallography NMR software (30) interspersed with model building in O-6 (31) yielded the complete structure. Although their electron densities were clear, the novobiocin molecules were only included at the last stages of the refinement. Anisotropic scaling and a bulk solvent correction were used and individual B atomic factors were refined anisotropically. Before a last refinement step, solvent molecules were added and individual B atomic factors were refined anisotropically. The 43K ATPase domain is 48% identical to the *E. coli* enzyme with 62% similarity in this domain (Fig. 1). The structure of the *T. thermophilus* A2B2 complex are similar to the *E. coli* A2B2 assembly and probably a greater stability of the individual subunits.

**RESULTS**

**The T. thermophilus Gyrase B Presents the Same Activity and the Same Response to Coumarin Inhibition as the E. coli Enzyme**—We chose the *T. thermophilus* enzyme because of the sequence conservation with the already known *E. coli* system from which structural and enzymatic data are available and to take advantage of its thermostable properties. The *T. thermophilus* Gyrase B shares 51% identity and 64.6% similarity with the *E. coli* enzyme. The two enzymes essentially differ due to the presence of an additional stretch of about 165 amino acids between residues 559 and 728 in the *E. coli* sequence (15). This insertion domain is missing in Gram-positive bacteria and mycoplasma, it is also absent in eukaryotic topoisomerase II.

| Table I Enzymatic activities of *E. coli* and *T. thermophilus* A2B2 enzymes |
|---------------------------------------------------------------|
| Enzymatic activity | *E. coli* A2B2 | *T. thermophilus* A2B2 |
|---------------------|---------------|----------------------|
| Supercoiling        | ++            | ++                   |
| Decatenation        | ++            | ++                   |
| Novobiocin inhibition | 1:40     | 1:47                 |
| Optimum temperature | 37 °C         | 65 °C                |
| Temperature range   | 16–47 °C      | 25–77 °C             |

Nevertheless, the catalytic activity data obtained for the *T. thermophilus* A2B2 complex are similar to the *E. coli* A2B2 one (Table I). The thermophile enzyme presents the same response as the *E. coli* protein to the inhibition by novobiocin.

The catalytic sites of the *E. coli* and *T. thermophilus* gyrase are very similar. The 43K ATPase domain is 48% identical to the *E. coli* enzyme with 62% similarity in this domain (Fig. 1). The limits of the *T. thermophilus* 43K domain were engineered by comparison with the *E. coli* sequence which led to the crystallographic determination of its structure (see “Experimental Procedures”). The recombinant protein was overproduced in *E. coli* and purified to more than 95% purity using two chromatographic steps (details of the protocol will be published elsewhere).

**The T. thermophilus GyrB 43K Domain Is a Dimer in the Presence of Novobiocin**—We incubated the N-terminal 43K domain (residues comprising the initial Met to residue 392) in the presence of an excess of novobiocin prior to crystallization. Thin plates of less than 10 μm thick appeared at 295 K using sodium formate as the precipitating agent. This crystal form could be reproducibly produced at this temperature and also at 278 K. The crystals belong to the space group P2₁ (a = 44.8 Å, b = 125.5 Å, c = 79.8 Å, β = 96.3°) with 2 molecules per asymmetric unit. The crystals were grown in a cryo-1 loop (Hampton Research) using a cryo-1 solution of 15–10% PEG8000 and 5% sodium formate as a precipitating agent. This crystal form could be reliably reproduced at this temperature and also at 278 K. The crystals belong to the space group P2₁ (a = 44.8 Å, b = 125.5 Å, c = 79.8 Å, α = γ = 90°, β = 96.3°) with 2 molecules per asymmetric unit and diffract to 2.3 Å. The 43K N-terminal of *T. thermophilus* GyrB presents the same fold as the *E. coli* enzyme with two structural subdomains (11). All residues from Ala¹ could be constructed out of the density map, the 8 extreme N-terminal residues being disordered. In both proteins, a novobiocin molecule could be localized at the entry of the ATP binding pocket, partially covering its binding site (see below).

In the crystal, the *T. thermophilus* 43K-novobiocin complex forms a dimer similar to the one previously observed between the *E. coli* enzyme with ADPFFNP, one monomer being related to the other by a noncrystallographic 2-fold axis. The superposition of monomer A with monomer B of the *T. thermophilus* dimer shows a root mean square deviation of 0.55 Å, which indicates that the two molecules are identical.

The existence of a dimer in the presence of novobiocin was not expected according to previous data obtained in the field. The structure of the *E. coli* 24K domain in complex with novobiocin showed a monomeric structure (24). This could be explained because many residues involved in the dimeric contacts in domain 2 were not present in this construct. Furthermore, the characterization of the oligomeric state of the *E. coli* GyrB by gel filtration chromatography and analytical ultracentrifugation showed that the 43K-novobiocin complex behaves mainly like a monomer in solution (33).

We performed gel exclusion chromatography experiments on

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1. L. Hoermann et al., unpublished data.
2. V. Lamour et al., unpublished data.
the entire GyrB in the absence and presence of novobiocin and ADPPNP. In the Sepharose gel, the protein behaves as a monomer when alone or in the presence of novobiocin and as a dimer in presence of the ADPPNP (data not shown). To analyze the species present in the elution fractions, we examined an aliquot of the top of each peak by native gel electrophoresis. This confirms that the GyrB alone is a monomer whereas both monomer and dimer coexist in the presence of ADPPNP or novobiocin (Fig. 2). Nevertheless, the fraction of dimeric species is more important for the ADPPNP-GyrB complex than for the novobiocin-GyrB complex, suggesting that the dimeric form with novobiocin is much less stable than the one with ADPPNP. The same experiments were performed on the 43K domain and showed similar results indicating that most of the dimeric contacts lie in the presence of ADPPNP or novobiocin (Fig. 2). Nevertheless, the fraction of dimeric species is more important for the ADPPNP-GyrB complex than for the novobiocin-GyrB complex, suggesting that the dimeric form with novobiocin is much less stable than the one with ADPPNP. The same experiments were performed on the 43K domain and showed similar results indicating that most of the dimeric contacts lie in the presence of ADPPNP or novobiocin (Fig. 2). Indeed, the buried surface in the novobiocin dimer interface (2141 Å$^2$) is 20% smaller than that of the E. coli-ADPPNP complex (2710 Å$^2$), a fact that could explain the decreased stability of this complex in solution.

Moreover, in the complex with ADPPNP, Tyr$^5$ makes an interaction which contributes to the stabilization of the dimer. In our structure, the presence of novobiocin prevents this residue from closing the ATP binding pocket, further decreasing the stability of the dimer. Nevertheless, this interaction was shown to be dispensable for the formation of the dimer whereas mutation of Ile$^{10}$ alone strongly affects the stability of the 43K domain to dimerize (6). Despite the equilibrium between the monomeric and the dimeric forms of the 43K-ADPPNP complex in solution, the previous crystallographic structures showed that the dimeric form was stabilized in the crystal (6, 11). In the crystallization conditions we used, we could also stabilize the dimeric form of the 43K domain in complex with novobiocin.

The T. thermophilus Dimer Exhibits Conformational Differences with the E. coli Dimer—In both E. coli and T. thermophilus dimers, there are three regions of interaction between the molecules forming the 43K dimer. The two C-terminal helices and loops 274–286 make few Van der Waals contacts, whereas the N-terminal part of each monomer contributes to most of the contacts with elements of the ATP-binding site. Both E. coli and T. thermophilus dimers occupy roughly the same volume (Fig. 3a). However, the superposition of domain 1 of a monomer of the T. thermophilus complex on domain 1 of
the E. coli complex leads to a root mean square deviation of 3.6 Å suggesting a large rearrangement of the dimer interface (Fig. 3b). We can observe that the second molecule has rotated about 18° around an axis positioned between the monomers defining a different orientation from the position it adopts in the E. coli complex. As a consequence, the buried surface between the monomers is reduced and the residues that are involved in the dimeric contacts of the E. coli complex constitute a subset of those participating to the dimeric interface of the ADPPNP complex.

At the level of one monomer, the superposition of domain 1 of one E. coli monomer on one of the T. thermophilus complex shows a root mean square deviation of 2 Å and reveals a large movement of domain 2 (Fig. 3c), whereas the ATP-binding region remains unchanged except loop 98–118 (see below). The spatial movement of domain 2 takes root in the hinge region of Gly220 and Gly221. The new position of domain 2 is resumed as a rotation of 18° around an axis containing Gly221.

**Implication of Loop 98–118 in Both Dimer and Domain 1/Domain 2 Conformations—**The most important dimeric contacts are localized between the loop 98–118 closing the ATP-binding site of one monomer and the first 30 N-terminal residues of the other monomer. The β-sheet formed by the N-terminal residues 10 to 13 from one monomer with the loop residues 98 to 100 from the other monomer is maintained. Nevertheless, compared with the ADPPNP complex, this loop embraced a wider volume (Fig. 4a). In its N-terminal part, the new position of the loop induces a slight bending of loop 78–90 due to the strictly conserved Gly78, so as to keep the dimeric contact between Asp45 and Lys14 from the other monomer. The other loop residues implicated in the dimeric contacts are rearranged to stabilize the new dimeric interface. Histidines A99 and A116 form a water mediated hydrogen bond with TyrA26. The symmetrical interaction network can be found in the other monomer between TyrA26, HisA108, HisA115, and a water molecule (Fig. 4b). In the E. coli complex with ADPPNP, TyrA26 is in direct interaction with HisA108 and with the main chain carbonyl group of HisA116, whereas the side chain of HisA116 is implicated in another hydrogen bond network with the side chain of Gln335.

In addition, the loop 98–116 appears to play a key role in the relative position of domain 2 versus domain 1. This loop forms two contacts with domain 2: Ser111 (E. coli Ser112) interacts with Asn271 whereas Tyr108 (E. coli Tyr109) makes a hydrogen bond with Glu249. Although maintained, these contacts are slightly rearranged due to the new conformation of loop 98–118 in the T. thermophilus complex. The interactions between the conserved residues His116, Tyr26, and Glu335 are lost in the absence of ADPPNP and the contact between Gly113 and Lys337 implicated in the binding of the γ-phosphate of ADPPNP is also...
lost due to the position adopted by loop 98–118 in this region (see Fig. 4a).

The conformation adopted by loop 98–118 is different from the one observed with ADP/PNP. Its position is stabilized by both dimeric interactions and contacts with domain 2 which results in rearrangements in both dimer interface and orientation of domain 2.

Novobiocin Binding to the 43K Domain of T. thermophilus Gyrase B—The novobiocin molecule is maintained at the entry of the active site by both hydrophobic and hydrophilic contacts, partially overlapping the ATP-binding site. The strongest interactions of the coumarin with the ATP binding pocket as the one with the conserved Arg136, are the same as those observed in the crystal structure of the E. coli 24-kDa domain with novobiocin (24). One additional strong hydrogen bond with the side chain of Asp80 (a glycine in the E. coli sequence) can be observed whose implications will be discussed below. Some weak interactions with novobiocin are slightly modified compared with the E. coli 24-kDa novobiocin complex. The carboxylic side chain oxygen of Glu49 is at a H-bond distance of the novobiose ring whereas in the E. coli 24 kDa-novobiocin complex, the corresponding Glu50 binds the novobiose ring through an ordered water molecule.

Interestingly, the stabilization of loop 98–118 by dimeric contacts allows additional interactions of the loop residues with the novobiocin molecule which could not be seen in the 24 kDa-novobiocin complex due to the monomeric structure of domain 1 alone. There are three additional hydrophobic contacts with residues Phe103, Val117, and Ile10 from the other monomer with novobiocin and some additional water-mediated contacts with the main chain of Tyr98 and Val117 (Fig. 5).

Furthermore, Lys102 and Lys109 point toward the novobiocin molecule suggesting weak interactions with the inhibitor (Fig. 5). In the E. coli complex, loop 98–118 is wrapping tightly the ADP/PNP molecule in a conformation where Lys 102 binds the /H9252 phospho-phosphate of ADP/PNP. We can also observe that Tyr108 which positions the ATP adenine ring points outside the active site in the presence of novobiocin. Positioning of the Tyr108 side chain inside the pocket would result in a steric clash with the novobiocin.
DISCUSSION

Inhibitor Binding to the T. thermophilus Gyrase B 43K Domain—The hydrogen bond between Asp$^{80}$ and the novobiocin molecule constitutes a specific feature of the novobiocin binding to the T. thermophilus enzyme. In E. coli, this residue is replaced by a glycine. At this position the gyrase family exhibits either a negatively charged residue (Asp or Glu) or an hydrophobic residue which is mainly a glycine, and sometimes an alanine in the prokaryotic GyrBs (15). In a novobiocin-resistant strain of Haloferax, this residue is replaced by a glycine while at the same time Ser$^{121}$ and Arg$^{136}$ are also mutated (34). Arg$^{136}$ is a strictly conserved residue and its only mutation provokes novobiocin resistance in various organisms (34–36). Up to now, the mutation of Asp$^{80}$ alone was not shown to induce novobiocin resistance but it is worth noting that the eukaryotic TopIIs sequences present a valine at this position, suggesting that the novobiocin binding specificity for the bacteria possessing a charged residue may partly lie in this region.

As for Asp$^{80}$ in the resistant strain of Haloferax, the mutation of Ser$^{121}$ (Ser$^{120}$ in T. thermophilus) alone does not induce novobiocin resistance and in the E. coli complex with ADPPNP, this residue makes no particular interactions with other residues. However, in the present conformation of the dimer, loop 98–118 is stabilized by an internal water-mediated hydrogen bond network between residues from its N and C terminus ends implicating Ser$^{120}$. A mutation at this position could affect the stability of the loop and destabilize this particular conformation of the catalytic pocket and as a consequence the novobiocin-binding site.

“Closed” and “Open” Conformations: Insights into the ATP Hydrolysis Conformational Implications—In the structure of the E. coli-ADPPNP complex, the conserved residues among the type II topoisomerases, Lys$^{337}$ and Gln$^{335}$ are within hydrogen bonding distance of the $\gamma$-phosphate (6, 11). Tyr$^{108}$ induces the bending of loop 98–118 over the ATP molecule by interacting with the adenine ring, whereas Asn$^{46}$ links the $\alpha$-phosphate. Lys$^{102}$ contributes to the positioning of the $\beta$-phosphate with the conserved Glu$^{11}$ (Glu$^{12}$ in E. coli) which is involved in the nucleophilic attack of the ATP through a Mg$^{2+}$ ion (37). The C-terminal part of loop 98–118 together with residues Gln$^{335}$ and Lys$^{337}$ of domain 2, is very tightly closed on the $\gamma$-phosphate (Fig. 4a), this can be considered as a closed conformation of the active site.

The new conformation of loop 98–118 is essentially stabilized by strong dimeric contacts and interactions with domain 2, leaving most of the ATP-binding site filled with water molecules. The novobiocin molecule has trapped an open conformation of the active site in contrast with the closed conformation adopted upon ATP binding.

The hydrolysis of ATP bound in the closed conformation and the departure of the reaction products would induce a complete reorganization of the active site by provoking the breaking of the hydrogen bonds between the $\beta$-phosphate and Lys$^{337}$ (E. coli Lys$^{336}$), between His$^{116}$ and Gln$^{335}$, as well as between the main chain amide group of Tyr$^{108}$ (E. coli Tyr$^{109}$) and the adenine ring. In the open conformation, the side chain of Lys$^{102}$ is directed to the inside of the active site whereas those of Lys$^{102}$ and Tyr$^{108}$ point outside the active site, in the opposite direction from the one they adopt in the closed conformation (Fig. 6). The flexibility of the two conserved pairs of glycine upstream and downstream loop 98–118 would facilitate the rearrangement of the backbone in the absence of ATP. Previous mutational studies of Lys$^{102}$, Lys$^{109}$ and Tyr$^{108}$ were shown to impair the catalytic activity of type II topoisomerases (16, 17). These data all together with the structural information from both open and closed conformations correlate the catalytic activity of the enzyme to the motions of the loop closing the active site.

Moreover, mutagenesis studies showed that Lys$^{337}$ has a critical role in the ATPase reaction in both prokaryotic and eukaryotic organisms and it was suggested that this residue is likely to be implicated in the stabilization of the transition state (12, 13). In the conformation we observe, Gln$^{335}$ and Lys$^{337}$ are pushed away by the dilatation of loop 98–118 which fills the space left free by the $\beta$- and $\gamma$-phosphates and leads to the complete swivel of domain 2. Furthermore, the breaking of the hydrogen bond between Gln$^{335}$ and Tyr$^{108}$ involved in dimeric contacts would initiate the conformational changes observed at the dimeric interface. These results are in agreement with previous works suggesting that Gln$^{335}$ and Lys$^{337}$ provide a mechanism for signaling the hydrolysis of ATP to the rest of the protein by initiating conformational changes following ATP hydrolysis (11).

The relative orientation of domain 1 and 2 and the resulting dimeric interface are coupled to the loop 98–118 conformation adopted upon ATP hydrolysis. Since gyrase B can bind ADP while keeping a partial activity (38, 39), there should be a particular conformation of the catalytic site in the presence of ADP. The conformation adopted right after ATP hydrolysis, in the presence of ADP and after the release of inorganic phosphate, may be an intermediate conformation between the closed and the open conformation and may define another dimer interface.

CONCLUSION

The catalytic activity of the T. thermophilus enzyme is identical to that of the E. coli enzyme with the advantages of its thermostable properties. The large homology of the T. thermophilus gyrase with the E. coli enzyme allows us to use the structural observations for understanding the function. This enzyme provided a tool for observing easily other reaction intermediates. Our results show that the novobiocin molecule has trapped a new dimeric conformation of the ATP-binding 43K domain. Considering the implication of the loop residues in the stabilization of the new conformation of the catalytic pocket, this clarifies the origin of some otherwise unexplained resistance mechanisms. In particular, mutation of Ser$^{121}$ could decrease the occurrence of the open conformation and thus, together with the other mutations, prevent novobiocin from binding. Furthermore, the description of the full 43K active site provide additional information about possible contacts with the compa-
rins which could be used to design more specific compounds.

The coumarins and in particular novobiocin are naturally occurring compounds found in certain strains of Streptomyces (40). In the case of the EF-Tu complex of *T. thermophilus*, the presence of the natural aurodox antibiotics stabilizes the GTP-induced conformation of the active site despite of the presence of GDP (41). Combined with enzymatic studies, our structure strongly suggests that the conformation trapped by novobiocin represent an open conformational state adopted by the enzyme immediately after ATP hydrolysis and product release. It suggests how conformational changes are driven by ATP hydrolysis at the level of the active site. This structure together with the analysis of the available enzymatic data emphasize the crucial role of Lys$^{337}$ and Gln$^{335}$ in the transmission of conformational changes upon ATP hydrolysis. Furthermore, this structure gives new insights into the implication of the loop 98–118 residues Lys$^{102}$, Tyr$^{109}$, and Lys$^{110}$ in the mechanism of release of the ATP molecule.

The recent crystallographic structures of yeast topoisomerase II have pointed out the possible relative positions of the different domains leading to a model for the translocation of DNA through the distinct sites of the enzyme (4). However, these structures do not contain the ATPase domain and the mechanism of coupling DNA translocation with ATP hydrolysis awaits further structural data. The present structure provides some clues on how the subdomains of the ATPase region undergo considerable conformational changes which are propagated from the active site and may be governed by the ATP turnover.

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