

Evidence for Multiple Functionality of *Campylobacter jejuni* DNA Gyrase in Chromosomal Replication

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ABSTRACT

Background: Bacterial topoisomerases are attractive targets for the development of novel antibiotics. DNA Gyrase and Topoisomerase IV (Topo IV) play essential roles in managing the topological state of DNA during bacterial chromosome replication. The molecular complexes responsible for modifying the topological state of DNA are heterotetramers, comprising GyrA and GyrB in Gyrase, and ParE and ParC in Topo IV. Despite performing distinct roles in DNA replication, the protein components of DNA Gyrase and Topo IV are highly conserved. Unlike most pathogens, *Campylobacter jejuni* lacks the genes that encode Topo IV, suggesting that the DNA gyrase complex is capable of performing multiple functions in DNA replication. Here we show that DNA gyrase performs both DNA supercoiling as well as chromosomal decatenation functions.

Methods: The *gyrA* and *gyrB* genes from *C. jejuni* ATCC 33560 were cloned, expressed and purified. Enzymatic activities of the holoenzyme and the individual subunits were determined using an ATPase assay, gel based supercoiling and decatenation assays.

Results: The ATPase activity of the GyrA/GyrB complex increased as compared to GyrB only. ATPase activity was not stimulated by the addition of DNA and could be modulated by salt and Mg^{2+} concentrations. ATPase activity was abolished by novobiocin. Gel based assays showed that the reconstituted holoenzyme (A_2B_2) was capable of supercoiling relaxed circular DNA as well as decatenating kDNA minicircles

Conclusions: The heterotetrameric gyrase from *C. jejuni* exhibits activities similar to previously characterized bacterial gyrases. However, the ability for the enzyme to efficiently decatenate circular DNA at physiologically relevant conditions is a likely explanation as to why this organism has been able survive without the typically essential *parE/parC* genes.

INTRODUCTION

Under contract with the National Institute of Allergy and Infectious Diseases (NIAID), we are developing small molecule drugs targeting the highly conserved ATP binding domains of DNA Gyrase and Topoisomerase IV. These enzymes are independently essential, but can simultaneously be targeted by inhibitors. Curiously, the important Biodefense pathogen *Campylobacter jejuni* does not contain *parC* and *parE*, which encodes Topo IV, suggesting that the Gyrase complex may perform multiple roles in DNA replication. To test this hypothesis, we have characterized the recombinant DNA gyrase from *C. jejuni*. ATPase, DNA supercoiling and decatenation activities of *C. jejuni* Gyrase have been examined and preliminary results are presented here.

METHODS

- Protein Purification:** Recombinant Gyrase from both *E. coli* and *C. jejuni* were purified by expressing individual subunits as hexahistidine fusions in pET28/BL21 (DE3) followed by IMAC and Ion Exchange Chromatography. All subunits were dialyzed to a final buffer of 25mM Tris 7.5 50mM NaCl 2mM DTT.
- ATPase Assay:** Enzymatic hydrolysis of ATP to ADP plus Pi was coupled to the conversion of 2-amino-6-mercapto-7-methylpurine riboside to ribose 1-phosphate and 2-amino-6-mercapto-7-methylpurine by purine nucleoside phosphorylase¹. The increase of absorbance was monitored at 360 nm for thirty minutes.
- Gel Based Assays:** Kit based supercoiling and decatenation assays were employed according to standardized protocols².

RESULTS

Overlay of the ATP-binding Domains of *E. coli* GyrB and *C. jejuni* GyrB.

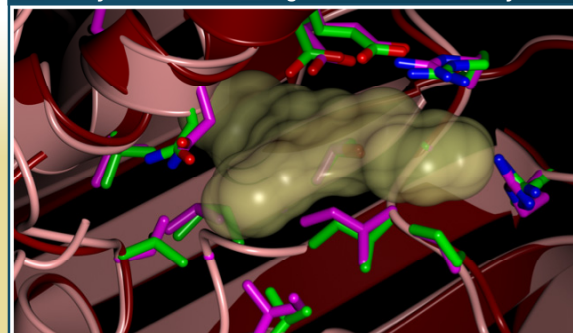


Figure 1. Comparison of the structures of the ATPase domains of *E. coli* GyrB (pink ribbons, magenta side-chains) and *C. jejuni* GyrB (brown ribbons, green side-chains). A transparent surface representation of a Trius inhibitor bound to the *E. coli* enzyme is shown for context. The two enzymes are structurally and compositionally highly similar, with conservation of all key ATP-binding residues. The 24 kDa ATP-binding domains overlay with an r.m.s.d. of <0.7 Å across all atoms.

ATPase Activity of *C. jejuni* DNA Gyrase

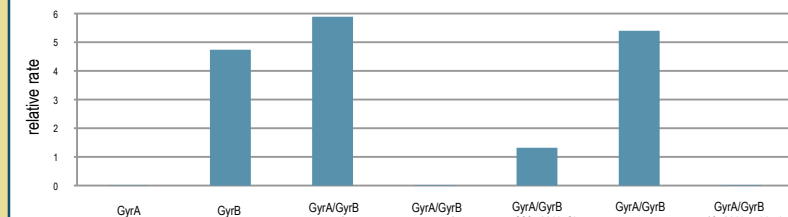
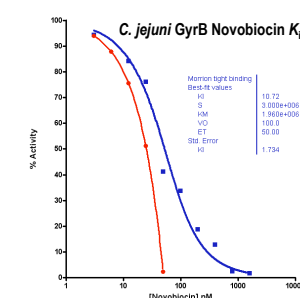


Figure 2. Relative ATPase kinetics of *C. jejuni* subunits, A+B and additives. ATPase activity was stimulated by the addition of equimolar GyrA. The rate was not stimulated by the addition of relaxed pBR322 DNA. The activity was greatly inhibited by 200mM NaCl and was completely abolished by the addition of 10µM Novobiocin.

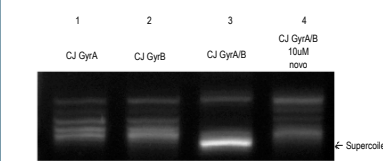
Novobiocin Inhibition of GyrB and ParE Enzymes



GyrB	Novobiocin K_i (nM)
<i>C. jejuni</i>	10.7
<i>F. tularensis</i>	0.14
<i>E. coli</i>	5.8
<i>E. faecalis</i>	9.2
ParE	
<i>E. coli</i>	81
<i>E. faecalis</i>	1072

Figure 3. Inhibition of ATPase activity by Novobiocin is similar to other GyrB enzymes. Enzymatic rates were plotted against a serial dilution of inhibitor, and the data was fit to the Morrison equation for tight binding inhibition. Red = theoretical limit of inhibition

Supercoiling Activities of *C. jejuni* Gyrase



Prior to running gel based topoisomerase assays, *E. coli* (EC) and *C. jejuni* (CJ) holoenzymes were normalized by Bradford assay², relative ATPase rates, and active site titration with a tight binding inhibitor. Both enzymes exhibited similar kinetic parameters, consistent with literature values.

Figure 4. Gyrase subunits in isolation lacked DNA supercoiling ability (1&2), and the reaction was completely inhibited by Novobiocin (4).

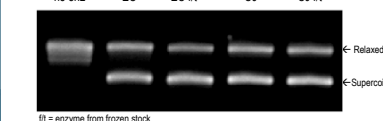


Figure 5. Equimolar EC and CJ gyrase exhibit comparable supercoiling ability under test conditions. The maximal no effect concentration of Novobiocin was within two fold for both enzymes which correlates well with the observed K_i data.

C. jejuni DNA gyrase is an Efficient Decatenase

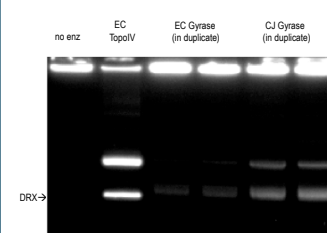


Figure 6. The *C. jejuni* enzyme is a more efficient decatenase of kDNA networks to minicircles than *E. coli* gyrase. The reactions were run under identical conditions normalized by protein concentration, ATPase activity and supercoiling as shown above.

E. coli topoisomerase IV (tested equimolar to the gyrases) was included to show the relative efficiency of decatenation compared to that of gyrase(s). Note that TopoIV does not supercoil closed circular decatenated DNA. Catenated kDNA remains in the gel wells.

Modulating the Supercoiling of Decatenated DNA

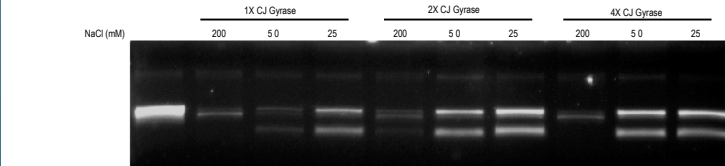


Figure 7. The decatenation and subsequent supercoiling of DNA minicircles could be modulated by different salt concentrations. At 200mM NaCl supercoiling of decatenated DNA is inhibited. At the low enzyme concentrations, modest and high salt levels reduced both activities.

RESULTS & DISCUSSION

- DNA Gyrase from *C. jejuni* has been expressed in *E. coli*, purified to homogeneity, characterized structurally, enzymatically and for supercoiling/decatenation activities.
- The ATP binding domain of the *C. jejuni* enzyme is structurally and compositionally conserved when compared to the well characterized *E. coli* enzyme. The K_i for Novobiocin against *C. jejuni* GyrB is in line with other gyrases, suggesting that broad spectrum GyrB inhibitors should retain activity against the *C. jejuni* enzyme.
- The *C. jejuni* gyrase has comparable ATPase kinetics and supercoiling activity when compared to the *E. coli* enzyme, but unlike the *E. coli* enzyme, also functions as an efficient decatenase.
- Inhibitors targeting the ATP binding pocket of the *C. jejuni* enzyme could disrupt both supercoiling and decatenation. However, drugs targeting this enzyme may be more susceptible to the development of target based resistance due to the lack of dual targeting.
- The ability to differentially modulate supercoiling and decatenation activities *in vitro* suggests that these activities may be regulated by cellular conditions *in vivo*.

CONCLUSIONS

- C. jejuni* DNA gyrase is a multifunctional topoisomerase, possessing both supercoiling and decatenation activities.
- The ATPase kinetics and inhibition by Novobiocin of *C. jejuni* gyrase are similar to *E. coli* gyrase and other bacterial pathogens.

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REFERENCES

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