DNA gyrase can cleave short DNA fragments in the presence of quinolone drugs

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ABSTRACT

We have analysed the DNA cleavage reaction of DNA gyrase using oligonucleotides annealed to a single-stranded M13 derivative containing a preferred gyrase cleavage site. We find that gyrase can cleave duplexes down to ~20 bp in size in the presence of the quinolone drugs ciprofloxacin and oxolinic acid. Ciprofloxacin shows a variation in its site specificity with an apparent preference for G bases adjacent to the cleavage sites, whereas oxolinic acid stimulates cleavage predominantly at the previously determined site. With either drug, cleavage will not occur within 6 bases from the end of a DNA duplex or a nick. We suggest that cleavage site specificity with short DNA duplexes is determined by drug–DNA interactions whereas with longer fragments the positioning effect of the DNA wrap around gyrase prescribes the site of cleavage.

INTRODUCTION

DNA gyrase is the enzyme from bacteria which can introduce supercoils into closed-circular DNA using the free energy of ATP hydrolysis (1,2). Gyrase is a member of a group of enzymes called DNA topoisomerases which are involved in the control of DNA topology (3). These enzymes can be divided into two types: type I enzymes catalyse reactions involving transient single-strand breaks in DNA while type II enzymes break both strands of the duplex. DNA gyrase is a type II enzyme and shares significant sequence similarity with other enzymes of the group (4). While many type II enzymes favour intermolecular topoisomerase reactions (e.g. decatenation), gyrase strongly favours intramolecular reactions and is the only enzyme able to catalyse the introduction of supercoils into DNA.

The mechanism of DNA supercoiling by gyrase involves the following steps: (i) the binding of the enzyme to DNA and the wrapping of a segment of DNA (~130 bp) around the enzyme in a positively-supercoiled sense; (ii) cleavage of this wrapped DNA (the G-segment) in both strands, involving the formation of covalent bonds between the 5’-phosphates at the break sites and specific tyrosines in the enzyme; (iii) passage of another segment of DNA (the T-segment) through this break; (iv) resealing of the break. Catalytic supercoiling requires the hydrolysis of ATP but in the presence of the non-hydrolysable ATP analogue ADPNP (5’-adenylyl β,γ-imidodiphosphate) limited supercoiling can be achieved, indicating that nucleotide binding will promote one round of supercoiling and that hydrolysis is required for the enzyme to turnover.

DNA gyrase from Escherichia coli consists of two proteins, GyrA and GyrB, of molecular masses 97 and 90 kDa respectively; the active enzyme is an A2B2 complex. The gyrase proteins have been shown to be organised as functional domains (1,2). The GyrA protein consists of an N-terminal domain (59–64 kDa) which contains the active-site tyrosine (Tyr122) for DNA cleavage and has interactions with the quinolone drugs, and a C-terminal domain (33 kDa) involved in the wrapping of DNA around the A2B2 complex. The GyrB protein consists of an N-terminal domain (43 kDa) which hydrolyses ATP and binds the coumarin drugs, and a C-terminal domain (47 kDa) which binds to the A protein and DNA. The structure of the 43 kDa N-terminal domain complexed with ADPNP has been solved to 2.5 Å resolution by X-ray crystallography (5). The structure of a 59 kDa N-terminal fragment of GyrA has also recently been solved (6).

Gyrase is the target of a number of antibacterial agents many of which belong to the quinolone and coumarin classes (7). Coumarin drugs inhibit supercoiling by preventing the hydrolysis of ATP by gyrase (8–10). The structure of an N-terminal sub-domain of GyrB (24 kDa) complexed with novobiocin has recently been determined (11). These data and recent binding experiments (12) support the idea that coumarins are competitive inhibitors of ATP binding. Quinolones (e.g. oxolinic acid and ciprofloxacin) inhibit DNA supercoiling by stabilising the complex between gyrase and cleaved DNA (8,9,13). Incubation of gyrase and DNA in the presence of a quinolone drug and denaturation of the complex leads to double-strand cleavage of the DNA with a 4-base stagger between the sites on the two strands. Specifically a tyrosyl-phosphate ester is formed between Tyr122 of GyrA and the 5’-phosphates of the protruding single-strand termini. The GyrA protein, which is covalently attached to the DNA, may be removed by digestion with proteinase K to reveal the broken DNA (14,15). DNA cleavage by gyrase can also be revealed in this way in the absence of quinolones when Ca2+ is substituted for Mg2+ in the reaction (16). Quinolone-induced DNA cleavage by gyrase has been shown to be reversible by treatment with EDTA or heat prior to the addition of denaturant (14,15). Although the supercoiling reaction of gyrase requires both subunits, DNA cleavage can be carried out by the N-terminal domain of GyrA complexed with GyrB (16,17); the smallest GyrA fragment which can carry out this reaction is ~59 kDa. Recently it has been shown that a 59 kDa...
N-terminal GyrA fragment complexed with GyrB can carry out ATP-dependent relaxation and decatenation in reactions reminiscent of more conventional type II enzymes (18).

The ability of quinolone drugs to trap a complex in which gyrase is covalently attached to broken DNA is strong evidence for the existence of a protein–DNA covalent intermediate in the supercoiling cycle. This reaction is common to all topoisomerases and a number of drugs can stabilise a cleavable complex in this way. In the case of the eukaryotic topoisomerase II (topo II; the counterpart to gyrase in higher organisms), a number of anti-tumour drugs (e.g. etoposide, amsacrine) have been shown to stabilise such a complex (19,20).

Although the gyrase/quinolone cleavage reaction is well documented, there is relatively little known about its detailed mechanism. In the case of the eukaryotic enzymes (topo I and II) this reaction has been studied using suicide substrates. In this case short DNA molecules have been designed such that cleavage by the enzyme yields a product which cannot be religated, allowing the separation of the cleavage and religation half reactions (21–24). In the case of gyrase this approach is not readily applicable, as the enzyme normally binds a stretch of DNA covalently attached to broken DNA is strong evidence for the existence of a protein–DNA covalent intermediate in the supercoiling cycle. This reaction is common to all topoisomerases and a number of drugs can stabilise a cleavable complex in this way. In the case of the eukaryotic topoisomerase II (topo II; the counterpart to gyrase in higher organisms), a number of anti-tumour drugs (e.g. etoposide, amsacrine) have been shown to stabilise such a complex (19,20).

This result is consistent with earlier observations of the stabilising effects of quinolones on the gyrase–DNA complex (31).

In this paper we have explored the use of short DNA fragments in the DNA gyrase cleavage reaction. Specifically we have cloned the preferred gyrase cleavage site from plasmid pBR322 (25,32,33) into bacteriophage M13 DNA and probed the DNA cleavage reaction by annealing oligonucleotides to the single-stranded form of this recombinant M13 DNA.

**Materials and Methods**

### Cloning

To construct M13-147, double-stranded M13mp18 DNA (34) was digested with SmaI and treated with calf-intestinal phosphatase [Boehringer Mannheim (35)]. A 147 bp fragment containing the preferred gyrase cleavage site from pBR322 was excised from plasmid pSTD147 (36) by digestion with AvaI. The ends were blunted using Klenow DNA polymerase (New England BioLabs) and dNTPs and the fragment ligated with linearised M13mp18 using T4 DNA ligase (Gibco-BRL). The ligation mixture was transformed into competent XL1-Blue cells. The presence of a single copy of the 147 bp insert was confirmed by restriction enzyme analysis and by DNA sequencing.

### Enzymes and DNA

The gyrase A and B proteins were purified as described by Hallett et al. (37). The 59 kDa N-terminal GyrA fragment (gift of Ms C.V. Smith) was prepared as described previously (17). Negatively supercoiled and relaxed forms of plasmid pBR322 were prepared as described previously (38). Linear pBR322 was prepared by digestion of the supercoiled form with EcoRI. M13 double-stranded (ds) DNA was prepared as described by Sambrook et al. (35).

Single-stranded (ss) M13 DNA was prepared as described by Sambrook et al. (35) except that an additional purification step was introduced to separate the ssDNA from small quantities of sheared chromosomal *E. coli* DNA present. The DNA was resuspended in 10 mM Na2HPO4/NaH2PO4 buffer (pH 7.2) and applied to a 5 ml Econo-Pac™ hydroxyapatite column (Bio-Rad). The column was washed with 10 mM phosphate buffer, and the ssDNA and dsDNA were eluted with a gradient of 10–500 mM phosphate buffer at the same pH. Single-stranded DNA eluted at a concentration of ~200 mM phosphate while the chromosomal DNA eluted at ~300 mM phosphate. The eluted samples were de-salted with NAP-25™ columns (Pharmacia) and the DNA precipitated with ethanol.

Oligonucleotides (oligos) were synthesised by Dr K.S. Lilley (University of Leicester) and repurified from polyacrylamide gels (35). Oligos were 5′-end labelled using T4 polynucleotide kinase (Gibco-BRL) and [γ-32P]ATP in ‘1-phor-all plus’ buffer (Pharmacia).

### Enzyme assays

DNA supercoiling reactions were carried out as described previously (16), under the following conditions: 35 mM Tris–HCl (pH 7.5), 24 mM KCl, 4 mM MgCl2, 5 mM dithiothreitol, 6.5% (w/v) glycerol, 1.8 mM spermidine, 1.4 mM ATP, 0.36 mg/ml BSA, 9 µg/ml tRNA, 10 µg/ml relaxed pBR322 DNA; incubations were for 1 h at 25°C. DNA cleavage assays were performed in the presence of either 0.2 mM CFX or 1 mM oxolinic acid (OXA) under supercoiling reaction conditions with the exception of an incubation time of 45 min at 25°C. Reactions were stopped by the addition of 0.2% SDS and 0.1 mg/ml proteinase K and the incubation continued for 30 min at 37°C (16,17,39). Cleavage reactions in the presence of Ca2+ contained 4 mM CaCl2 in place of MgCl2. ATPase assays were carried out as described previously (40).
RESULTS

Construction of an M13 derivative containing a preferred gyrase cleavage site

In order to prepare ssDNA molecules containing a known gyrase cleavage site, a 147 bp DNA fragment from pBR322 containing the preferred quinolone-induced cleavage site, at bp 990 on the top strand, was cloned into M13. This site has been extensively studied previously (25, 29, 33, 36). The M13 derivative was named M13-147.

The rates of supercoiling of relaxed M13-147 and M13 were compared in a time course with gyrase and found to be identical (data not shown), i.e. the introduction of an additional gyrase binding site made no significant difference to the rate of supercoiling. To determine the principal sites of gyrase cleavage in dsM13-147 and dsM13, an oxolinic acid-directed cleavage assay was carried out on the linear form of both substrates. From the sizes of the resulting products, it was possible to determine the approximate sites of gyrase cleavage. We found that the principal cleavage product in both cases originated from a site within M13, indicating that a strong gyrase cleavage site is already present in M13. However, the pattern of bands produced indicated that the cloned site was cleaved secondarily. Since M13 is about twice the size of pBR322, and given the weak sequence specificity of the gyrase cleavage reaction, it was not surprising to find that a strong cleavage site was already present in M13. The exact position of the strong cleavage site was not determined. Since the pBR322 '990' site has been extensively studied we continued to use this site for further work. This experiment indicates that the pBR322 990 site is not the strongest site for quinolone-induced gyrase cleavage; indeed other stronger sites are known to exist (41–43).

When ssM13-147 was used as a substrate in CFX-induced gyrase cleavage reactions, no cleaved products were found. When ssM13-147 was used as a cofactor in gyrase ATPase reactions, no stimulation of the ATPase activity was seen, consistent with previous observations (44). By contrast, in the presence of dsM13-147, gyrase showed the expected level of DNA-dependent ATPase activity (data not shown). These results confirm that gyrase shows no detectable interaction with ssDNA.

Cleavage of partially dsDNA substrates

Having obtained an M13 derivative containing a preferred gyrase cleavage site in single-stranded form, it was now possible to anneal a range of oligonucleotides to the region containing the preferred site and determine whether they were cleavage substrates for gyrase. To show that such partial duplexes can be cleaved in this context, a 150-base oligo was made which spanned the entire 147 bp sequence. Using radiolabelled 150mer, cleavage stimulated by oxolinic acid and ciprofloxacin was shown to occur (data not shown). Figure 1 shows the range of shorter oligonucleotides used in subsequent experiments. In each case the labelled oligo was annealed to ssM13-147 (top strand) and used as a substrate for the gyrase cleavage reaction in the presence of oxolinic acid and ciprofloxacin. Using oligo R39 the conditions for the cleavage reaction were optimised. With 2.1 nM ssM13-147, 2-fold excess labelled oligo and excess gyrase (42 nM), we found that optimal levels of cleavage occurred with ~0.2 mM CFX and ~1 mM OXO. These levels of drug and enzyme are ~10-fold higher than those required with larger duplex substrates and may reflect a decreased stability of the complex between gyrase and the short double-stranded regions. As discussed below this is a likely consequence of weaker gyrase–DNA interactions in complexes in which there is interaction with only the central (cleaved) portion of the wrapped segment. During time-course experiments we found that maximal levels of cleavage occurred after ~30 min with the partially double-stranded substrates under these conditions.
Gyrase cleavage reactions using the above conditions were carried out with the oligos shown in Figure 1. Example autoradiographs of the results from these cleavage experiments are shown in Figure 2 and the results are summarised in Table 1. We found that all oligos >20 bases were cleaved at a similar level by gyrase (Fig. 2). F20 was cleaved less efficiently, and F19, F18 and F18* showed little or no cleavage. Smaller oligos (F16 and F12) showed no detectable cleavage. Although the site of cleavage was similar with all oligos there was some variation (Fig. 3), with the predominant cleavage site being different for the two drugs. In the presence of OXO the major cleavage site was at the expected 990 position, but with CFX the major site was 4 bases away at base 994 on the top strand (Fig. 3). In the case of CFX a number of secondary cleavage sites were also noted. It was found that cleavage would not occur when the site was ≤ 6 bp from the end of a fragment. For example, with F26 the major CFX site is no longer cleaved as it is now 6 bp away from the end of the oligo. Six different CFX cleavage sites around the 990 site have been observed in this study (Fig. 3), four of which are between GG dinucleotides in one of the strands (see Discussion). Although it seems that gyrase can cleave DNA duplexes down to ~20 bp in size in the presence of quinolones, we were unable to detect cleavage stimulated by Ca²⁺; for example R39 shows no detectable cleavage products in the presence of Ca²⁺. This suggests important differences between the stabilisation of the cleavable complex by quinolones and Ca²⁺.

It is feasible that the results with the partially double-stranded substrates might be influenced by flanking sequences, i.e. if the single-stranded part of M13-147 helps to stabilise the gyrase complex. Therefore selected cleavage reactions were carried out using double-stranded versions of the oligonucleotides (Table 1). We found that the results were consistent with those obtained using the partially double-stranded substrates.

**Cleavage reactions with the 59 kDa N-terminal domain of Gyra**

The DNA cleavage activity of DNA gyrase has been shown to reside within the N-terminal domain of Gyra (16) and fragments derived from the N-terminal part of Gyra, when complexed with Gyrb, have been shown to cleave DNA in the presence of quinolones as efficiently as the intact enzyme (17). Using selected oligos (down to F24) we found that the cleavage reaction was identical to that with the intact enzyme (e.g. Fig. 4). This result implies that with these substrates the 33 kDa domain of Gyra, which is involved in DNA wrapping (45), does not influence the cleavage reaction and that, in these experiments, we are observing the interaction between DNA and the DNA breakage–reunion domain of Gyra (see Discussion).

**Suicide substrates**

A fruitful approach to probing the DNA cleavage reactions of topoisomerases and other enzymes has been the use of ‘suicide’ substrates (21,46,47). These substrates are constructed such that, following cleavage, religation is disfavoured so that cleaved intermediates can be isolated. With eukaryotic topo II, suicide substrates can be made with short oligonucleotides (e.g. 17 bp).
both drugs the enzyme can cleave substrates down to ∼20 bp in the presence of oxolinic acid. Surprisingly, we find that in the presence of the quinolone drugs ciprofloxacin to the region containing the preferred cleavage site, and have oligonucleotides, between 12 and 150 bases long, which anneal DNA gyrase. We have prepared a series of complementary stranded form this modified M13 does not appear to interact with plasmid pBR322 into M13. We have shown that in its single- have cloned the preferred quinolone-induced cleavage site from In order to probe the DNA cleavage reaction of DNA gyrase we DISCUSSION

In order to probe the DNA cleavage reaction of DNA gyrase we have cloned the preferred quinolone-induced cleavage site from plasmid pBR322 into M13. We have shown that in its single-stranded form this modified M13 does not appear to interact with DNA gyrase. We have prepared a series of complementary oligonucleotides, between 12 and 150 bases long, which anneal to the region containing the preferred cleavage site, and have assessed the ability of gyrase to cleave these partial duplex substrates in the presence of the quinolone drugs ciprofloxacin and oxolinic acid. Surprisingly, we find that in the presence of both drugs the enzyme can cleave substrates down to ∼20 bp in size. In other work (e.g. footprinting and ATPase experiments) it was found that gyrase interacts with DNA duplexes of ≥100 bp (25–29,48). However, drug-induced cleavage with the quinolone fleroxacin has been found with a 71 bp molecule (30), although oxolinic acid-induced cleavage of a 34 bp duplex, based on the pBR322 990 site (33), was found not to occur. We find that a very similar 34 bp substrate and indeed smaller substrates (Table 1) are in fact cleaved. These differences may be due to differing reaction conditions. Gmünder et al. (49) have also recently shown that gyrase can cleave DNA duplexes as short as 20 bp in the presence of quinolone drugs.

We found that DNA cleavage stimulated by Ca2+ did not occur with the short duplexes (<39 bp), consistent with the work of Gmünder et al. who showed that Ca2+ cleavage did not occur within an 85 bp fragment (30). However, Ca2+ can also induce cleavage of linear DNA is not catalysed by the N-terminal 64 kDa domain of GyrA (in the presence of GyrB) unless the C-terminal 33 kDa wrapping domain is also present (50). Again these results suggest important differences in the quinolone- and Ca2+-induced cleavage of DNA by gyrase.

It is well known that when gyrase binds DNA a section of ~130 bp is wrapped around the enzyme. This consists of a central portion of 13–40 bp (25–29) and flanking regions. It is likely that the central portion interacts with the N-terminal domain of GyrA containing the DNA breakage–reunion site whilst the flanking portions interact with the 33 kDa C-term ‘wrapping’ domain of GyrA. This is borne out by recent experiments which shows that the complex between the 159 kDa N-terminal domain of GyrA and GyrB shows no DNA wrapping (18). In addition, transcriptional footprinting of gyrase in the presence of CFX indicates that ~20 bp of DNA surrounding the 990 cleavage site is inaccessible to T7 RNA polymerase (51). Thus, we suggest that the binding of gyrase to DNA consists of two elements, a central segment within which DNA cleavage occurs and flanking regions involved in DNA wrapping. It would appear that, for quinolone-induced cleavage, binding to only the central portion is required. It is interesting to note that the limit size of DNA fragments cleavable by gyrase is very similar to the size of fragments that can be cleaved by topo II. For example Lund et al. (52) have shown that calf thymus topo II can cleave a duplex of 16 bp. This is also consistent with the fact that when topo II binds DNA it cleaves but does not wrap and therefore has a correspondingly smaller footprint than gyrase (∼25 bp (53)).

Although we found that cleavage induced by oxolinic acid occurred at the expected 990 site, cleavage induced by ciprofloxacin occurred at a number of positions with 994 being the preferred site
likely to arise with such fragments, as seen in Figure 3. We
active site in any orientation. Hence a range of cleavage sites
exerts a preferred rotational orientation on the bound DNA and
the 59 kDa domain (Fig. 4). In earlier work using longer DNA
molecules (147 and 198 bp) drug-induced cleavage was found to
occur almost exclusively at the 990 site (29,36). This suggests
that the DNA wrap, mediated by the 33 kDa domains of GyrA,
exerts a preferred rotational orientation on the bound DNA and
thus prescribes the site of cleavage. It is likely that preferred sites
are those where the flanking DNA has an intrinsic curvature or
higher than average flexibility. In the experiments described in
this paper the constraints imposed by DNA wrapping are not
present and the short fragments are free to bind in the enzyme’s
active site in any orientation. Hence a range of cleavage sites is
likely to arise with such fragments, as seen in Figure 3. We
suggest therefore that in these experiments cleavage sites may be
determined largely by drug–DNA interactions.

In the work of Gmünder et al. (49), the principal cleavage sites
with fleroxacin and ciprofloxacin were found to be at bp 990 and
993 in the top strand, compared with 990 and 994 in our work
(Fig. 3). It is not clear why these sites are not identical but it may
reflect different reaction conditions and the variability of cleavage
sites with fluoroquinolones. It is worth noting in this regard that
in previous work by Gmünder et al. (30) quinolone-induced
cleavage by gysrane of DNA fragments based on the 990 site
occurred at 990 and 994. In this work cleavage of the top strand
was monitored which supports the assertion that the cleavage sites
we have observed are indeed double-stranded breaks with a
4-base stagger between sites on opposite strands (see below).

In the experiments described in this paper only bottom strand
cleavage was generally followed, the bottom strand being the
labelled oligo and the top strand being intact single-stranded
M13-147 (Fig. 3). The assumption that cleavage also occurred in
the top strand at the expected locations is supported by several
considerations. Firstly, cleavage of DNA by gysrane to generate a
double-stranded break with a 4-base stagger has been widely
observed (25,26,54,55). Secondly, the two major sites we
observe in the bottom strand (990 and 994) have also been
independently observed in the top strand by Gmünder et al. (30).
Thirdly, we have carried out control experiments using double-
stranded F28 labelled in either the top or bottom strand and
observed cleavage products from both strands (data not shown).
The cleavage sites favoured by CFX seemed to show some
sequence preferences. Looking at the bases either side of the point
of cleavage in the top and bottom strands in the six CFX sites near
990 (Fig. 3), there seems to be a preference for G bases. All sites
have a purine–purine dinucleotide in at least one strand, and in four
out of the six sites cleavage occurs between a GG dinucleotide in
one strand. Previous work has suggested a preference for
quinolone binding to G residues and it is possible that the data in
this paper also reflect this preference for binding to Gs, perhaps
indicative of drug–DNA base interaction. For example, nalidixic
acid has been shown to bind to guanine on ssDNA in the presence
of copper ions (56,57), and norfloxacin showed a preference for
single-stranded G-containing homopolymers (58). Gmünder et al.
(49) found that gysrane-catalysed cleavage in the presence of
floroxacin and CFX showed a preference for a G residue 5′ to the
cleavage site in at least one strand. However, a detailed study of
sequence preferences would require the analysis of a number of
different gysrane cleavage sites.

Another interesting feature of drug-induced cleavage from this
work is that cleavage fails to occur at sites which are within 6 bp
of the end of a duplex (Fig. 3). This might suggest that there is an
important enzyme–DNA contact, ∼6–7 bases away from the
cleavage site, which is required for cleavage. Therefore, in theory,
gysrane should be capable of cleaving a duplex which is 18 bp in
length, with the cleavage site in the centre. In support of this
suggestion we find that F18* is cleaved weakly by gysrane at the
site in the centre of the duplex region (Fig. 1 and Table 1). It is
therefore unlikely that gysrane would cleave a duplex region <18 bp
in length, because cleavage would have to occur within 6 bases
of either end of the duplex. This is distinct from eukaryotic topo
II which can cleave a substrate with only 3 bp on one side of the
cleavage site (21).

In the work of Gmünder et al. (49) a ladder of products formed
by cleavage close to the ends of their substrate DNAs was found.
The formation of these products was Mg2+- dependent and the
activity was present with GyrA or GyrB alone. The authors
speculate that this activity is due to non-specific cleavage activity
of gysrane or the presence of a co-purifying nuclease. Our work
tends to favour the latter explanation. Our initial experiments also
revealed ladders of cleavage products which we found to be due to
a Mg2+-dependent activity associated with GyrB. As our GyrB
had no intrinsic supercoiling activity it was unlikely to be
contaminated with GyrA. We found that the laddering could be
substantially reduced by using an altered purification procedure
involving a GyrA-affinity column (59). Evidence presented
above also argues against gysrane cleaving at the ends of DNA
substrates because of the requirement for at least 6 bp on each side
of the cleavage site.

One of the aims of this work was to develop substrates suitable
for suicide cleavage reactions. The observations of cleavage
of short duplexes suggested that this approach might be feasible.
However, although a number of suicide substrates were designed
based around F36 and a number of cleavage conditions explored,
we were unable to find evidence of suicide reactions. [Failure of
gysrane to cleave suicide substrates has also been noted in another
lab (H. Gmünder, personal communication)]. One reason for this
is the requirement for at least 6 bases either side of the cleavage
site, discussed above. The fact that substrates with >6 bases either
side but containing a nick within the 6 bases were not cleaved
might imply that the requirement reflects a structural transition in
the DNA which occurs at the cleavage site. The recently
determined crystal structure of the 59 kDa N-terminal domain of
GyrA (6) suggests that distortion of the DNA near the cleavage
site is likely to occur, i.e. the presence of a nick near the cleavage
site may prevent distortion of the DNA required for cleavage.

In summary, these experiments show that gysrane can cleave
DNA duplexes down to ∼20 bp in size but cannot cleave at a site
which is >6 bp from the end of a fragment. The quinolone drug
CFX shows preferences for G bases adjacent to the cleavage site,
which may reflect drug–base interaction.

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