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Micrococcus luteus DNA gyrase: Active components and a model for its supercoiling of DNA

(positive superhelical DNA/DNA topoisomerase)

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ABSTRACT **Two active components α and β of *Micrococcus luteus* DNA gyrase, of peptide weights of 115,000 and 97,000, respectively, have been purified. Each individual component exhibits little DNA gyrase activity; the ATP-dependent negative supercoiling of a covalently closed circular DNA duplex is catalyzed by a combination of the two. Covalent closure by *Escherichia coli* ligase of a circular DNA containing single-chain scissions, when carried out in the presence of a combination of the DNA gyrase components α and β, gives a positively supercoiled DNA upon removal of the bound protein molecules. ATP was not present during the ligase treatment; therefore the positive supercoiling of DNA observed is a result of the binding of gyrase molecules, presumably as multi-subunit oligomers, during the ligation step. This is in contrast to the negative supercoiling of DNA catalyzed by gyrase in the presence of ATP. A model in which negative supercoiling of DNA is achieved by ATP-modulated repetitive wrapping of the DNA around gyrase is described. The model also suggests a plausible mode of action by which translocation of a DNA along its helix axis can be actively driven by an ATPase.**

A novel *Escherichia coli* enzyme, termed DNA gyrase, has been discovered recently (1). This enzyme converts a covalently closed circular DNA without superhelical turns to a highly negatively supercoiled form. In other words, the enzyme causes a reduction of the linking number (2) of the covalently closed circular duplex. The reaction requires ATP, the hydrolysis of which presumably provides the driving force for the otherwise energetically unfavorable reaction. The ATP-dependent negative supercoiling of DNA promoted by gyrase is phenomenologically opposite to the ATP-independent removal of negative superhelical turns by *E. coli* ω protein [*Eco* DNA topoisomerase I (3)].*

In vitro and *in vivo* studies indicate that in *E. coli* two widely separate genetic loci are involved in gyrase activity. These are the locus *cou*, which determines resistance to two related antibiotics (coumermycin A and novobiocin), and the locus *nalA*, which determines resistance to another two related drugs (nalidixic and oxolinic acids) (4–6). Gyrase purified from cells sensitive to these antibacterial agents is inhibited by them in its supercoiling of DNA, whereas the same enzyme purified from mutant cells resistant to these agents is not (4–6). Because *in vivo* these drugs block DNA replication (7–9) and the supercoiling of superinfected λ DNA (4–6), the identification of gyrase as the target of these drugs in turn indicates that gyrase is involved in DNA replication and supercoiling in *E. coli* (1, 4, 6).

E. coli gyrase has been purified by a number of groups (1, 5, 6, 10). The *nalA* gene product has been purified to homogeneity (5). It appears to have a DNA topoisomerase activity distinct from *Eco* DNA topoisomerase I (5).

In this communication, we report our studies on two active components, α and β, of *Micrococcus luteus* gyrase. The possibility that negative superhelical turns in a DNA might be introduced through ATP-modulated wrapping of the DNA *around* gyrase will be discussed.

MATERIALS AND METHODS

Chemicals and Enzymes. Novobiocin, nalidixic acid, ATP, thioglycolic acid, and spermidine were purchased from Sigma. Coumermycin was kindly donated by Bristol Laboratories, and Polymin P was kindly donated by BASF. Purified *E. coli* and *M. luteus* DNA topoisomerase I and antisera against each protein were prepared as described earlier, (11, 12).

Methods. Nondenaturing polyacrylamide gel electrophoresis of proteins was done as described by Davies (13), using Tris-glycine buffer, pH 8.3, containing 0.06% thioglycolic acid. For two-dimensional gel electrophoresis, a nondenaturing 7.5% polyacrylamide cylindrical gel was run first. The gel was soaked in 1% sodium dodecyl sulfate/3% (vol/vol) 2-mercaptoethanol/75 mM sodium phosphate, pH 7.0/10% (wt/vol) glycerol, for 10 min at 80°. It was then placed horizontally on top of a sodium dodecyl sulfate/polyacrylamide (5%) slab, which had been cast in such a way that protein markers could be run in two slots flanking the cylindrical gel. Ethidium fluorescence assay of the degree of superhelicity of a covalently closed DNA (14) was done according to the high-pH procedure of Morgan and Pulleyblank (15), using a Perkin–Elmer model 43 spectrofluorimeter.

Purification of *M. luteus* Gyrase. The purification procedure will be published elsewhere. Briefly, the procedure involves lysis by lysozyme, Polymin P precipitation and extraction of the precipitate, ammonium sulfate fractionation, DEAE-cellulose column chromatography, and hydroxylapatite column chromatography. The components α and β separate at the hydroxylapatite step. Fractions containing α and those containing β were pooled separately, and were further chromatographed on phosphocellulose to give fraction VIa, which contains α, and fraction VIb, which contains β. Starting from 300 g of spray-dried *M. luteus* cells (Miles), about 6 ml of fraction VIa containing 0.9 mg of protein per ml, and 4.5 ml of fraction VIb containing 1.4 mg of protein per ml were obtained. Protein concentrations were measured according to the procedure of Bradford (16).

RESULTS

***M. luteus* Gyrase Has at Least Two Components.** When low amounts of fraction VIa and VIb were assayed separately,

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* We have proposed that enzymes that catalyze the *concerted* breaking and rejoining of DNA backbone bonds be referred to as DNA topoisomerases, because they are invariably detected and studied by their isomerization of different DNA topological isomers.

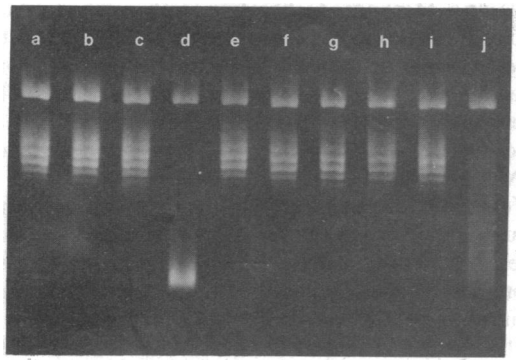

FIG. 1. ATP-dependent DNA supercoiling activity of *M. luteus* gyrase. Except omissions or additions noted for each sample, all reaction mixtures (70 μl each) contained 35 mM Tris·HCl (pH 8.0), 16 mM potassium phosphate, 4.4 mM spermidine, 1.4 mM $MgCl_2$, 1.4 mM ATP, 0.1 mM dithiothreitol, 0.2 mM Na_3EDTA, bovine serum albumin at 50 μg/ml, covalently closed circular PM2 DNA without superhelical turns (relaxed PM2 DNA) at 4.5 μg/ml, fraction VIb at 6.4 μg/ml, and fraction VIa at 4.9 μg/ml. After 30-min incubation at 37°, reactions were stopped by adding 15 μl each of 5% (wt/vol) sodium dodecyl sulfate, 25% (wt/vol) glycerol, and bromophenol blue at 0.25 mg/ml. Electrophoresis was carried out in a 0.7% agarose slab gel and in a buffer containing 40 mM Tris·HCl (pH 8.0), 5 mM Mg acetate, and 1 mM Na_3EDTA. Lane a, fractions VIa and VIb omitted; b, fraction VIa omitted; c, fraction VIb omitted; d, no omission; e, $MgCl_2$ omitted; f, spermidine omitted; g, potassium phosphate omitted; h, potassium phosphate omitted, 16 mM sodium phosphate added; i, dithiothreitol omitted, 700 μM 4-chloromercuribenzoate added; j, dithiothreitol omitted, 70 μM 4-chloromercuribenzoate added.

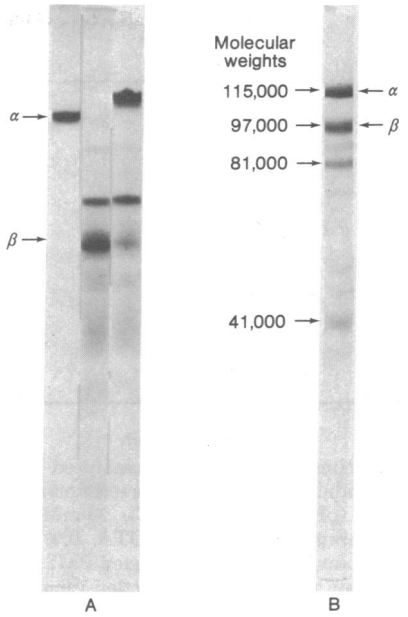

FIG. 2. (*A*) Nondenaturing polyacrylamide gel electrophoresis of *M. luteus* gyrase. After pre-electrophoresis at 4 mA per tube overnight at 4°, gels from left to right were loaded respectively with 5 μl of fraction VIa, 5 μl of fraction VIb, and 5 μl of fraction VIa plus 5 μl of fraction VIb. Electrophoresis was continued at 4° for 11 hr at a current of 0.75 mA per tube. A Bio-Rad tube gel electrophoresis apparatus was used. (*B*) Sodium dodecyl sulfate/polyacrylamide gel (10%) electrophoresis of a mixture of fractions VIa and VIb.

no DNA supercoiling activity was observable. Lanes a–c of Fig. 1 show, respectively, the gel electrophoretic patterns of control untreated relaxed DNA of phage PM2 and the same DNA after treatment with fraction VIa and VIb separately. The patterns are essentially identical. Supercoiling of the DNA occurred if the DNA was treated with a mixture of the two fractions (Fig. 1, lane d). When the amounts of proteins in the assays were increased by 10-fold over the amounts used in the samples shown in Fig. 1, lanes b and c, a very low level of supercoiling activity was detected in fraction VIa but not in fraction VIb. These results indicate that *M. luteus* DNA gyrase has at least two components contained separately in fraction VIa and fraction VIb, though fraction VIa probably contains a trace amount of the component in fraction VIb.

M. luteus Gyrase Requires K^+, Mg^{2+}, and ATP, and Is Inhibited by Novobiocin, Coumermycin, and Nalidixic Acid. *M. luteus* gyrase is similar to *E. coli* gyrase in its cofactor requirements and in its inhibition by a number of antibiotics. In addition to ATP, the DNA supercoiling reaction requires Mg^{2+} (Fig. 1, lane e), spermidine (Fig. 1, lane f), and K^+ (Fig. 1, lane g). Na^+ is completely ineffective in substituting for K^+ (Fig. 1, lane h). The spermidine requirement can be alleviated, however, by using a higher Mg^{2+} concentration. In the absence of spermidine the optimal Mg^{2+} and K^+ concentrations are 25 mM and 20 mM, respectively. The activity is inhibited by both novobiocin and coumermycin at a concentration of 1 μg/ml, and by nalidixic acid at concentrations >200 μg/ml (data not shown). It is also inhibited by sulfhydryl reagents 4-chloromercuribenzoate (Fig. 1, lanes i and j) and *N*-ethylmaleimide at a concentration of 10 mM (data not shown).

The α and β Components of _M. luteus_ Gyrase. The electrophoretic patterns of fraction VIa, fraction VIb, and an equal-volume mixture of the two in nondenaturing 7.5% polyacrylamide cylindrical gels are shown in Fig. 2*A*.

Fraction VIa gives a single band (leftmost gel in Fig. 2*A*). Assaying for activity complementing fraction VIb across an unstained gel, which was electrophoresed in parallel with the stained one, shows that the activity coincides with the position of the protein band. Electrophoresis of fraction VIa in a sodium dodecyl sulfate/polyacrylamide gel also gives a single band with a mobility corresponding to a peptide weight of 115,000. We designate this protein the α component of *M. luteus* gyrase.

Fraction VIb gives a number of bands (middle gel in Fig. 2*A*). To determine the peptide weights of the bands, a two-dimensional gel electrophoresis was carried out, as described in *Materials and Methods*. The most intense band in the middle gel shown in Fig. 2*A* has a peptide weight of 97,000. The sharp intense band above it has a peptide weight of 41,000. When slices of an unstained gel of fraction VIb, run in parallel with the stained one, were assayed for gyrase activity in the presence of added α the activity complementing α was found to coincide with the position of the most intense band. Thus it appears that the 97,000 peptide weight protein is the second component of *M. luteus* gyrase. We designate this protein the β component of the enzyme.

The identification of β as the second component is supported by its interaction with α. The rightmost gel pattern shown in Fig. 2*A* results when the same amounts of fraction VIa and VIb used for the two other gels are combined and run in parallel with the other gels. The intensity of the β band is greatly diminished; the α band appears to have slowed down. We have always observed the reduction of intensity of the β band by the addition of α. Analysis by two-dimensional gel electrophoresis indicates that the addition of α smears out the β band toward lower electrophoretic mobility.

Fig. 2*B* shows the electrophoretic pattern of a mixture of fractions VIa and VIb in a sodium dodecyl sulfate gel. The two most intense bands from the top down are α and β. We have already mentioned that the 41,000 peptide weight band cor-

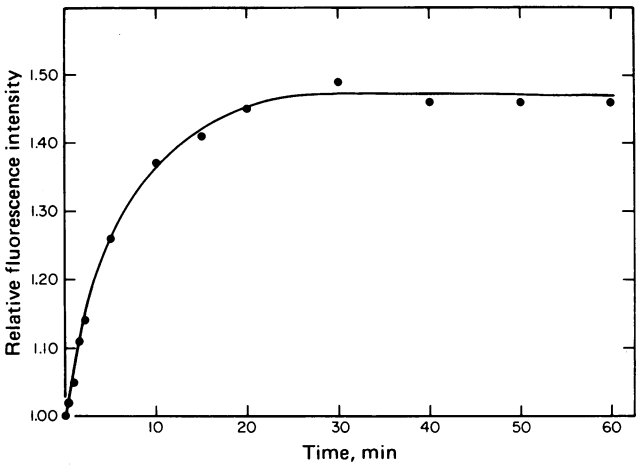

FIG. 3. The time course of gyrase-catalyzed DNA supercoiling monitored by ethidium fluorescence. The reaction mixture containing 50 mM Tris·HCl (pH 8.0), 25 mM $MgCl_2$, 20 mM KCl, 1 mM ATP, 0.2 mM dithiothreitol, 0.1 mM Na_3EDTA, 10% (wt/vol) glycerol, bovine serum albumin at 30 μg/ml, relaxed PM2 DNA at 20 μg/ml, fraction VIa protein at 1.6 μg/ml, and fraction VIb protein at 2.4 μg/ml was incubated at 23°. Aliquots (50 μl each) were taken out at time intervals and each was mixed with 1.2 ml of a solution containing 20 mM K_3PO_4 (pH ~12), ethidium bromide at 0.5 μg/ml, and 3 mM EDTA. The relative fluorescence of a sample is the ratio of the fluorescence intensity of the sample to that of a control without gyrase but otherwise identical. To start the reaction, two equal-volume solutions, one containing the DNA and the other gyrase, were incubated at 23° and mixed quickly at time zero.

responds to the sharp band above β in the nondenaturing gel. The 81,000 peptide band runs in the nondenaturing gel as a very broad band faster than β but smears into it. This species does not complement α to give gyrase activity, but we cannot rule out the possibility that it might be required for gyrase activity in addition to α and β. From the pattern shown in Fig. 2*B*, the amount of β in fraction VIb is estimated to be about 40% by mass.

Because gyrase promotes the ATP-dependent supercoiling of DNA, it must contain a DNA topoisomerase activity† and an activity that can hydrolyze ATP. The purified α component has no detectable DNA topoisomerase activity. The partially purified β component has a trace amount of DNA topoisomerase activity, but because a contamination of less than 0.1% of *M. luteus* ω protein (*Mlu* DNA topoisomerase I) would be sufficient to show this level of activity, it is difficult to assess whether β possesses an intrinsic topoisomerase activity. In the absence of ATP, there is no detectable topoisomerase activity that can relax positively supercoiled DNA in α, β, or a combination of α and β.

The purified α component (fraction VIa) has no detectable ATPase activity. Fraction VIb contains some ATPase activity, most of which appears to be due to a contaminant, because the bulk of the activity is neither DNA dependent nor inhibited by antibiotics that inhibit gyrase supercoiling activity. Further purification of β is necessary in order to study the DNA gyrase-related ATPase.

ATP-Dependent Negative Supercoiling of DNA Is Promoted by *M. luteus* Gyrase Catalytically. The time course of *luteus* gyrase-catalyzed supercoiling is illustrated in Fig. 3. The degree of superhelicity of the DNA substrate was monitored by the ethidium fluorescence procedure, as described in *Materials and Methods*. Calibration with a set of PM2 DNA samples of different degrees of superhelicity shows that the fluorescence intensity increases linearly with increasing number of negative superhelical turns per DNA molecule. The fluorescence intensity of the plateau region shown in Fig. 3 corresponds to a degree of superhelicity approximately equal to that of PM2 DNA extracted from the phage.

We define one unit of DNA supercoiling activity as the amount of gyrase that causes half of the maximal fluorescence increase in 30 min for 1 μg of PM2 DNA under the assay conditions described in the legend to Fig. 3. Results from a series of fluorescence assays with different amounts of gyrase indicate that the specific activity of an equal volume mixture of fractions VIa and VIb is about 50,000 units/mg of total protein. In other words, 20 μg of total protein, which contains approximately 70 fmol of α and 50 fmol of β, can cause the supercoiling of 1 μg or 150 fmol of PM2 DNA to half the maximal degree of superhelicity attainable in 30 min. In a separate experiment, 40 fmol of covalently closed PM2 DNA without superhelical turns was incubated with a mixture of fractions VIa and VIb containing 0.9 fmol of α and 0.6 fmol of β, for 22 hr at 23°. Gel electrophoresis of the product shows that about half of the DNA was converted to the highly negatively supercoiled form. These experiments show that *M. luteus* gyrase catalyzes the negative supercoiling of DNA in the presence of ATP; each enzyme molecule can convert a number of DNA molecules to the supercoiled form.

Covalent Closure of DNA in the Presence of Bound Gyrase Molecules Yields a Positively Supercoiled DNA. To test whether the *binding* of the *M. luteus* gyrase components affects the helical configuration of DNA, PM2 DNA samples containing one single-chain scission per DNA molecule were converted to the covalently closed form by *E. coli* DNA ligase in the absence and presence of one or both of the gyrase components. In order to avoid the gyrase-catalyzed negative supercoiling of the DNA after covalent closure by ligase, no ATP was added during ligation. We have shown in a separate experiment that the cofactor NAD, which is required for the *E. coli* ligase reaction, cannot substitute for ATP in the gyrase-catalyzed supercoiling of DNA. Following the covalent closure of the DNA samples by ligase, the relative linking numbers of the DNA were examined by gel electrophoresis after the removal of the bound proteins. The results are shown in Fig. 4.

Compared with the control DNA closed in the absence of α or β (Fig, 4, lane a), DNA covalently closed in the presence of α and β migrates much faster (Fig. 4, lane d). The component α itself causes a smaller increase in mobility (Fig. 4, lane c), and the component β and all other species in fraction VIb have no effect in the absence of α (Fig. 4, lane b). When decreasing amounts of mixtures of α and β are added during ligase treatment, a corresponding decrease in the shift of the mobilities of the group of covalently closed species is observed (Fig. 4, lanes e–g). The addition of coumermycin during ligase treatment has no effect on the mobility shifts of the products (Fig. 4, lanes h–l).

To determine whether the electrophoretic mobility shifts are due to negative or positive supercoiling of the DNA, electrophoresis of the same set of samples was carried out at a lower temperature (4°) and in a Mg^{2+}-containing buffer. The lowering of temperature and the addition of Mg^{2+} to the electrophoresis buffer increase the helix rotation (25), and therefore should decrease the number of positive superhelical turns or increase the number of negative superhelical turns. It is found that the mobilities of all covalently closed species, relative to the mobility of nicked PM2 DNA, are lower in the low-tem-

† We propose that the DNA topoisomerase activity of *M. luteus* gyrase be designated *Mlu* DNA topoisomerase II, and that of *E. coli* gyrase be designated *Eco* DNA topoisomerase II.

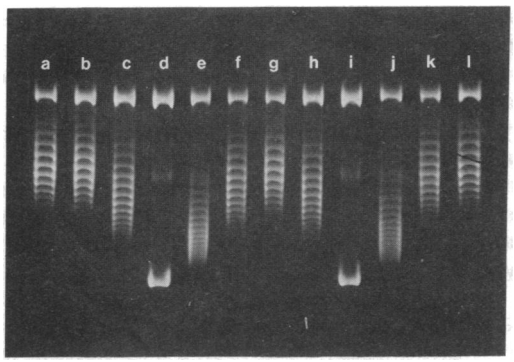

FIG. 4. Winding of the DNA double helix by *M. luteus* gyrase in the absence of ATP. Reaction mixtures (56 μl each) containing 43 mM Tris·HCl (pH 8.0), 21 mM $MgCl_2$, 11 mM potassium phosphate, 0.5 mM dithiothreitol, 0.2 mM Na_3EDTA, 5% (wt/vol) glycerol, bovine serum albumin at 42 μg/ml, NAD at 2.8 μg/ml, PM2 DNA with one single-chain scission per molecule at 13.4 μg/ml, and various amounts of fractions VIa and VIb were incubated at 23°. After 15 min, 6 μl of *E. coli* DNA ligase solution (1.7 units/ml in the same reaction buffer equilibrated at 23°) was added to each reaction mixture. Incubation at 23° was continued for another hour, and the reaction was terminated by the addition of 7 μl of a solution containing 5% (wt/vol) sodium dodecyl sulfate, 25% (wt/vol) glycerol, and bromophenol blue at 0.25 mg/ml. The mixture was loaded on a 0.7% (wt/vol) agarose slab gel. The electrophoresis buffer was 40 mM Tris·HCl (pH 8.0)/5 mM Na acetate/1 mM Na_3EDTA, and electrophoresis was carried out at room temperature. The amounts of gyrase fractions added to the samples are as follows: lane a, none; b, 2.9 μg of fraction VIb; c, 1.9 μg of fraction VIa; d, 2.9 μg of fraction VIb and 1.9 μg of fraction VIa; e, 0.72 μg of fraction VIb and 0.50 μg of fraction VIa; f, 0.18 μg of fraction VIb and 0.12 μg of fraction VIa; g, 0.045 μg of fraction VIb + 0.03 μg of fraction VIa; lanes h–l were the same as c–g, respectively, except coumermycin was present at 7 μg/ml in each sample during the treatment with ligase.

perature–Mg^{2+} gel than those of the corresponding bands in the gel shown in Fig. 4. This shows that all covalently closed species shown in Fig. 4 are *positively* supercoiled. In other words, covalent closure of a DNA in the presence of a combination of α and β but in the absence of ATP causes an *increase* in the linking number in proportion to the amount of protein added. It appears that α by itself can also cause an increase in the linking number, although we cannot rule out the possibility that this effect is due to the presence of a trace amount of β in fraction VIa.

DISCUSSION

Our results indicate that *M. luteus* DNA gyrase has two and probably only two components, α and β, of peptide weights 115,000 and 97,000, respectively. Although gyrase must possess a topoisomerase activity, α and β are distinct from *M. luteus* DNA toposiomerase I (12) in their sizes and chromatographic behavior.

The negative supercoiling of DNA by gyrase poses fascinating questions on the mechanism of the reaction. One mode of achieving negative supercoiling is by processes affecting the pitch of the DNA double helix. For example, DNA-dependent ATPases capable of disrupting base pairing of long segments of DNA are known (17–19). Such an activity, coupled with a DNA topoisomerase activity, could achieve the negative supercoiling of a covalently closed DNA.

We are led to consider a completely different mode of achieving negative supercoiling, however, by our finding that DNA covalently closed by ligase in the presence of gyrase has a higher linking number. This increase in linking number could be due to either a winding of the DNA helix by the binding of gyrase molecules or by the wrapping of the DNA *around* gyrase molecules. Although a number of proteins and small molecules are known to unwind the DNA helix (20–24), aside from small effects of counterions (25), winding of the DNA helix has not been observed. We therefore propose that the increase in linking number of a DNA covalently closed by ligase in the presence of DNA gyrase is due to the coiling of the DNA around the gyrase molecules. This type of DNA-wrapped-around-protein structure has been well documented in recent studies on the structure of the nucleosome in chromatin (26, 27).

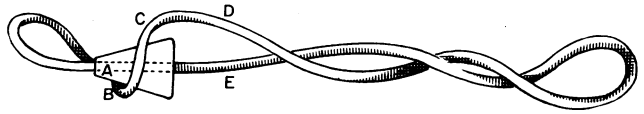

FIG. 5. One of the models in which negative supercoiling of the DNA is achieved by continued coiling of the DNA, modulated by ATP hydrolysis, around a gyrase molecule. For the particular model shown, the DNA passes through the enzyme, which could be achieved if the enzyme is a multi-subunit oligomer. The wrapping of the DNA *around* the enzyme in this particular model is left-handed. See footnote ‡ for a brief discussion on the handedness. Models that do not require the DNA to pass through the enzyme can also be constructed. Points A–E are discussed in the text.

The postulated wrapping of DNA around gyrase immediately points to the possibility that gyrase might achieve negative supercoiling of the DNA by continuously forming turns of the appropriate handedness around the enzyme. Many models can be conceived based on this mode of action. We wish to describe one model illustrated in Fig. 5, in order to discuss some general features.

According to this model, in the absence of ATP the DNA is bound to a gyrase molecule at point A in such a way that rotation of the DNA around its helical axis relative to the enzyme is forbidden. The DNA segment BC is wrapped around the enzyme with the appropriate handedness.‡ Experimentally, we know that if the DNA initially contains single-chain scissions, covalent closure of the DNA followed by the removal of bound gyrase gives a positively supercoiled DNA. Therefore, for a covalently closed DNA without superhelical turns in the absence of gyrase, wrapping of the segment BC around a bound gyrase results in negative supercoiling of the DNA outside the BC segment. Roughly speaking, one negative superhelical turn is introduced if the DNA is wrapped around the enzyme a full turn.

We now consider what might happen when ATP is added to the gyrase–DNA complex. With PM2 DNA as the substrate, in the presence of ATP the DNA after gyrase treatment contains about 100 negative superhelical turns per molecule. If the negative superhelical turns result from wrapping the DNA around the enzyme, it is necessary to wrap the DNA around the enzyme by about 100 turns. Clearly, at any given time the DNA can wrap around the enzyme by only a much smaller number of turns, say of the order of 1. We therefore postulate that the net effect of cycles of concerted conformational changes of the

‡ We cannot specify the handedness *a priori*. Suppose we start with a linear DNA, wrap a segment of it in a left-handed fashion around a hollow cylinder, then join the ends to convert it to the covalently closed form. The DNA is negatively superhelical upon removal of the cylinder. But if before joining the ends one end is first passed through the center of the cylinder, the resulting covalently closed DNA is positively superhelical upon removal of the cylinder. This inversion of the sense of superhelicity has been discussed in ref. 2. For the model shown in Fig. 5, the DNA does pass through the supercoiled region and therefore in this model the DNA is coiled around the enzyme left-handedly.

gyrase subunits, accompanying cycles of charging and discharging of the enzyme by ATP, is to produce a continued coiling of the DNA around the enzyme: when the lagging segment CD becomes wrapped around the enzyme (with the same handedness as before), the leading segment BC is displaced. The displacement of the segment BC means that the leading loop ABC becomes positively supercoiled. We postulate that the topoisomerase site of gyrase is located at A, so that the positive superhelical turns in the leading loop are readily removed. The net result of the ATP-dependent gyrase action is therefore the introduction of negative superhelical turns. In the next cycle of events, the segment CD becomes the leading segment, and the ATP-driven coiling process continues.

The question naturally arises on how the continued coiling of the DNA around the enzyme can be achieved by cycles of charging and discharging of the enzyme by ATP. One can imagine a simple case in which the enzyme is composed of two domains x and y. In the absence of ATP or when the enzyme is discharged, the DNA is wrapped around x. Charging of the enzyme produces concerted conformational changes of both x and y, resulting in the wrapping of the DNA around y and the release of the segment originally wrapped around x. This kind of charging–discharging cycles could produce the type of continued coiling we postulated.

The type of model illustrated in Fig. 5 has a number of interesting features. (*i*) The enzyme–DNA complex possesses an intrinsic handedness. Therefore, if two enzyme molecules are acting simultaneously on one DNA molecule, their effects cannot be destructive. (*ii*) The enzyme is interacting with a duplex DNA segment, and negative superhelical turns are generated without disruption of base pairing. (*iii*) The model illustrates one mechanism in which linear movement of a DNA relative to another macromolecule might be involved. This aspect is not an essential part of our model for the gyrase mechanism, however, For example, if the enzyme–DNA complex forms and dissociates readily, there need not be much linear displacement.

K. Mizuuchi and M. Gellert (personal communication) have proposed a model in which the gyrase sits at a crossing of two double helices, tightly bound to one double helix and making the other one translocate through the junction in an ATP-driven process. In their model the DNA chain always presents its same side (major groove, minor groove, or any other structural feature) to a given site on the gyrase responsible for the translocation. Thus, while DNA moves through the crossing, its helix turns do not. The results of such a translocation is to generate a negatively supercoiled loop on one side of the crossing and a positively supercoiled loop on the other side of the crossing. The topoisomerase site is located on the positively supercoiled loop side, thus leaving the DNA with a net negative supercoiling.

Our model shares a number of features with theirs. The basic difference between these two models lies in the mode of generating superhelical turns. In our model the superhelical turns are generated by coiling of the DNA around the gyrase. In the Mizuuchi–Gellert model the superhelical turns are generated by translocation of the DNA through a site on gyrase in such a way that the helical turns of the DNA duplex do not pass through the site.

Finally, we note that because of our finding that ligase closure in the presence of gyrase gives a positively supercoiled DNA, it is now possible to carry out physicochemical studies of positively supercoiled DNAs.

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