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Biochemistry

Energy coupling in DNA gyrase and the mechanism of action of novobiocin

(coumermycin A_1/oxolinic acid/site-specific DNA cleavage/DNA supercoiling/ATPase)

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ABSTRACT *Escherichia coli* DNA gyrase catalyzes negative supercoiling of closed duplex DNA at the expense of ATP. Two additional activities of the enzyme that have illuminated the energy coupling component of the supercoiling reaction are the DNA-dependent hydrolysis of ATP to ADP and P_i and the alteration by ATP of the DNA site specificity of the gyrase cleavage reaction. This cleavage of both DNA strands results from treatment with sodium dodecyl sulfate of the stable gyrase-DNA complex that is trapped by the inhibitor oxolinic acid. Either ATP or a nonhydrolyzable analogue, adenyl-5′-yl-imidodiphosphate (App[NH]p), shifts the primary cleavage site on ColE1 DNA. The prevention by novobiocin and coumermycin A_1 of this cleavage rearrangement places the site of action of the antibiotics at a reaction step prior to ATP hydrolysis. The step blocked is the binding of ATP because coumermycin A_1 and novobiocin interact competitively with ATP in the ATPase and supercoiling assays; the K_i values are more than four orders of magnitude less than the K_m for ATP. This simple mechanism accounts for all effects of the drugs on DNA gyrase. Studies with App[NH]p, another potent competitive inhibitor of reactions catalyzed by gyrase, show that cleavage of a high energy bond is not required for driving DNA into the higher energy supercoiled form. With substrate levels of gyrase, App[NH]p induces supercoiling that is proportional to the amount of enzyme; a −0.3 superhelical turn was introduced per gyrase protomer A. We postulate that ATP and App[NH]p are allosteric effectors of a conformational change of gyrase that leads to one round of supercoiling. Nucleotide dissociation favored by hydrolysis of ATP returns gyrase to its original conformation and thereby permits enzyme turnover. Such cyclic conformational changes accompanying alteration in nucleotide affinity also seem to be a common feature of energy transduction in other diverse processes including muscle contraction, protein synthesis, and oxidative phosphorylation.

DNA gyrase is an ATP-requiring bacterial enzyme that catalyzes negative supercoiling of closed duplex DNA (1–3). It is an essential function (4) implicated in DNA replication, transcription, and phage λ integrative recombination (1, 5). Gyrase is composed of two subunits that have been identified in preparations of the purified enzyme and have also been purified separately (3, 6). Subunit A of the *Escherichia coli* enzyme is the *nalA* gene product (molecular weight of protomer = 105,000) and determines sensitivity to the related pair of gyrase inhibitors, nalidixic acid (Nal) and oxolinic acid (Oxo) (6–9). Subunit B is specified by the *cou* locus (6) and determines sensitivity to another pair of drugs, novobiocin and coumermycin A_1 (7, 10, 11). Both subunits are required to reconstitute the five known activities of *E. coli* gyrase (6–8; unpublished data): (*i*) introduction of supercoils in the presence of ATP; (*ii*) relaxation of supercoils in the absence of ATP, a measure of the process we designate as "breakage and reunion" (B–R)[§] that is a component of all topoisomerases—enzymes that alter the linking number of DNA (12); (*iii*) DNA-dependent hydrolysis of ATP to ADP and P_i; (*iv*) enzyme binding to DNA; and (*v*) site-specific cleavage of DNA. The cleavage reaction was discovered during the study of the mechanism of action of Oxo (7, 8). Oxo inhibits gyrase by trapping a stable complex of enzyme and DNA that may be an intermediate in B–R analogous to intermediates proposed for other topoisomerases, such as *E. coli* ω protein (13) and the rat liver DNA untwisting enzyme (14). Treatment of the gyrase-DNA complex with sodium dodecyl sulfate ($NaDodSO_4$) results in double-strand cleavage of the DNA at specific DNA sequences and attachment of the denatured enzyme to the DNA by covalent bonds (7, 8). Cleavage, like B–R, is not inhibited by novobiocin or coumermycin A_1.

Gyrase is unique among topoisomerases in its ability to drive the DNA into the higher energy supercoiled form by using ATP as an energy source. This report focuses on energy transduction by DNA gyrase. We have found that novobiocin and coumermycin A_1 specifically poison energy coupling simply by preventing ATP binding. The nonhydrolyzable ATP analogue, adenyl-5′-yl-imidodiphosphate (App[NH]p), is also competitive with ATP and efficiently inhibits supercoiling and ATPase activities. However, this drug, mimicking ATP, promotes a change in the cleavage-site priority of gyrase. Indeed, simultaneous hydrolysis of ATP is not required for supercoiling because, with substrate levels of gyrase, App[NH]p induces supercoiling that is stoichiometric with the amount of enzyme. A model is presented in which ATP is an allosteric effector for a conformational change in DNA gyrase that drives one round of supercoiling; hydrolysis of the high energy anhydride bond in ATP is needed for enzyme turnover.

MATERIALS AND METHODS

Enzyme Assays. Gyrase was reconstituted from highly purified subunits A and B that were prepared as described (6). The 17-μl supercoiling assay mixture contained 35 mM Tris·HCl (pH 7.6), 18 mM potassium phosphate, 5 mM dithiothreitol, 10 mM $MgCl_2$, 5 mM spermidine·HCl, 50 μg of bovine serum albumin per ml, ATP or App[NH]p at the indicated level, and 70 fmol of relaxed ColE1 DNA molecules. Reactions were stopped by addition of 0.5% $NaDodSO_4$. The production of supercoiled ColE1 DNA was monitored by agarose gel electrophoresis in one of two ways. First, standard assays employed

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Abbreviations: App[NH]p, adenyl-5′-yl-imidodiphosphate; Nal, nalidixic acid; Oxo, oxolinic acid; $NaDodSO_4$, sodium dodecyl sulfate; B–R, breakage and reunion.

§ The term "breakage and reunion" is preferred to the more restricted designation, "nicking and closing," because the intermediate in relaxation by gyrase may contain a double-strand break rather than a nick in only one strand.

a 1% agarose gel at 23° as described (7). The amount of product was measured by microdensitometry of a photographic negative of gels stained with ethidium bromide. Second, where low numbers of superhelical turns were introduced, as in Figs. 4 and 5, both the standard agarose gel system and a 1% agarose gel run at 4° in a buffer containing 40 mM Tris·acetate (pH 7.8), 5 mM $MgCl_2$, and 1 mM EDTA were employed. With the latter conditions ColE1 DNA has about eight more negative superhelical turns (15) and the isomers of low negative supercoil density are well resolved. More highly supercoiled DNA is better resolved by the 23° gel system. Reaction extents were quantitated by counting the number of bands between the centers of mass of the starting material and the final product (15, 16). This method assumes that each band differs from its neighbors by a single superhelical turn and depends on resolving all the bands between the two populations to be compared. ATPase assays employed a supercoiling reaction mixture containing [γ-^{32}P]ATP (1000–4000 cpm/pmol). The release of $^{32}P_i$ was measured by chromatography with polyethyleneimine-cellulose thin layer plates from Brinkmann (17). Cleavage reactions used the supercoiling assay mixture except that 0.38 mM Oxo was included and the substrate was ColE1 DNA digested with *Eco*RI restriction endonuclease. After 60 min at 30°, 0.5% $NaDodSO_4$ and 60 μg of proteinase K (EM Biochemicals) per ml were added and incubation was continued for 15 min at 37°; proteinase K is neither necessary nor sufficient for cleavage but removes denatured enzyme bonded to the DNA and thereby sharpens agarose gel patterns.

Materials. ColE1 DNA was relaxed in a reaction mixture containing 40 mM Tris·HCl (pH 7.5), 0.2 M NaCl, 5 mM EDTA, and excess rat liver DNA untwisting enzyme; after 40 min at 37°, 0.1% $NaDodSO_4$ was added and the product was purified by CsCl/ethidium bromide density gradient centrifugation. App[NH]p (Boehringer Mannheim) was purified successively by polyethyleneimine-cellulose thin layer chromatography in 1 M HCOOH/0.5 M LiCl and by a DEAE-cellulose column developed with a linear 0–0.3 M triethylammonium bicarbonate (pH 8.0) gradient (18). It was free of detectable ATP contamination as monitored by paper chromatography in 1-propanol/concentrated ammonia/water, 6:3:1 (vol/vol) (18), and by the polyethyleneimine-cellulose system in which the R_F values for App[NH]p, ATP, App[NH], and ADP were 0.2, 0.1, 0.66, and 0.54, respectively. Novobiocin was from Sigma, coumermycin A_1 was a gift of K. Price (Bristol Laboratories), and Oxo was donated by J. D. Stein (Warner–Lambert Research Institute).

RESULTS

Alteration of DNA Gyrase Cleavage Pattern. Oxo traps a stable gyrase-DNA complex, treatment of which with $NaDodSO_4$ results in double-strand cleavage of DNA at specific sites (7, 8). When the substrate is full-length linear ColE1 DNA produced by digestion of the closed duplex form with *Eco*RI restriction endonuclease, the primary products resolved by agarose gel electrophoresis are two fragments designated a and a′ (Fig. 1, lane 2). They result from a single cleavage, 0.47 genome length (unpublished data) from the *Eco*RI cut end closest to the origin of replication (19). The less prominent bands in Fig. 1 result from cleavage at other sites on ColE1 DNA. It had been shown that the *amount* of cleavage of circular DNA was not significantly influenced by novobiocin or ATP (7); novobiocin (Fig. 1, lane 3) or coumermycin A_1 (data not shown) also did not affect the *distribution* of cleavage sites on the DNA. However, ATP altered the pattern dramatically (Fig. 1, lane 4). The a and a′ bands were diminished and bands b and b′ resulting from a single cleavage 0.41 genome length from the

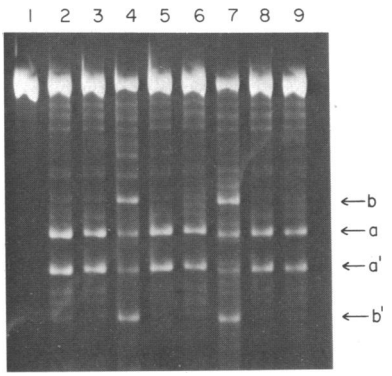

FIG. 1. The influence of drugs and nucleotides on the selection of gyrase cleavage sites. For assay of cleavage, we incubated standard 17-μl reaction mixtures containing *Eco*RI-restricted ColE1 DNA and 7.5 units (defined in ref. 6) of reconstituted DNA gyrase at 30° for 30 min to allow the enzyme to bind to DNA. After addition of drugs and nucleotides, incubation was continued for 30 min at 30° and the reactions were stopped by the addition of $NaDodSO_4$ and proteinase K to final concentrations of 0.5% and 60 μg/ml, respectively. DNA was displayed by agarose gel electrophoresis. Lane 1 shows the control without added drugs or nucleotide. Samples in lanes 2–9 contained 380 μM Oxo and either 0.4 μM novobiocin (lane 3), 500 μM ATP (lane 4), 500 μM ATP and 0.4 μM novobiocin (lane 5), 500 μM ATP and 0.4 μM coumermycin A_1 (lane 6), 500 μM App[NH]p (lane 7), 500 μM App[NH]p and 0.4 μM novobiocin (lane 8), or 500 μM App[NH]p and 0.4 μM coumermycin A_1 (lane 9).

*Eco*RI cut end closest to the origin of replication were significantly enhanced. This movement of the primary ColE1 DNA cut site by about 400 base pairs had the same high specificity for nucleotides as the fueling of supertwisting. The following compounds at a concentration of 1 mM did not alter the cleavage pattern or promote appreciable supercoiling: CTP, UTP, GTP, dATP, dCTP, dTTP, dGTP, AMP, and ADP. Novobiocin or coumermycin A_1 at 4×10^{-7} M blocked the alteration in cleavage pattern by 10^{-3} M ATP (Fig. 1, lanes 5 and 6) resulting in the same pattern as in the absence of ATP (Fig. 1, lane 2).

The results imply that transduction of chemical energy in the form of ATP through DNA gyrase can be monitored not only by supercoiling of closed duplex molecules but also by change of the primary site of cleavage on linear molecules. To determine if hydrolysis of ATP to ADP and P_i is required for this alteration in cleavage pattern, we tested the effect of the nonhydrolyzable analogue App[NH]p. The analogue changed the cleavage pattern in a manner very similar to ATP (Fig. 1, lane 7), and novobiocin or coumermycin A_1 abolished the change exactly as for ATP (Fig. 1, lanes 8 and 9). This suggests two important conclusions which are corroborated below. First, the binding of ATP is sufficient to alter gyrase cleavage specificity and thus at least a portion of energy transduction does not require the consumption of a high energy bond. Second, novobiocin and coumermycin A_1 prevent binding of ATP.

Novobiocin, Coumermycin A_1, and App[NH]p Are Competitive with ATP. If novobiocin and coumermycin A_1 simply prevent ATP binding, they must be competitive inhibitors. To test this, we measured the dependence of supercoiling (Fig. 2*A*) and of ATPase (Fig. 2*B*) activity on the concentration of ATP in the presence of several concentrations of novobiocin. Whereas supercoiling is the defining activity of gyrase, ATPase is easier to measure quantitatively. The intersection on the ordinate of the family of lines in the Lineweaver–Burk plots in Fig. 2 shows that ATP and novobiocin indeed interact competitively. The kinetic constants derived from the supercoiling and ATPase data, respectively, are 6.0×10^{-9} M and 1.0×10^{-8} M for the K_i for novobiocin and 2.5×10^{-4} M and 4.5×10^{-4}

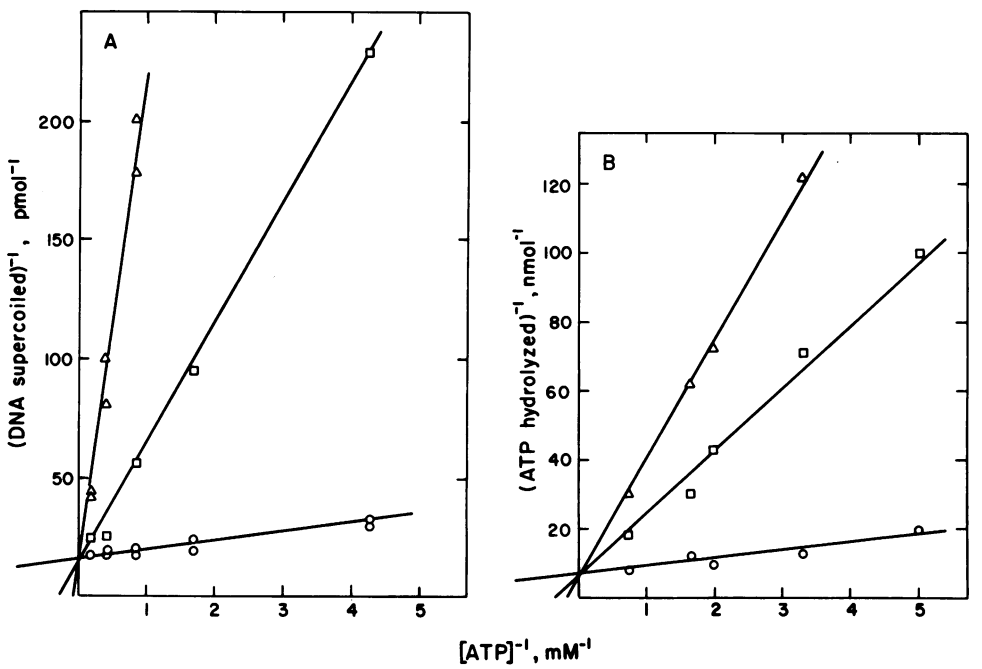

FIG. 2. Inhibition of DNA gyrase by novobiocin as a function of ATP concentration. (*A*) The supercoiling reaction mixtures contained 0.19 pmol of relaxed ColE1 DNA, the indicated amounts of ATP, 4 units of subunit A and 2 units of subunit B, and either no novobiocin (○), 97 nM novobiocin (□), or 290 nM novobiocin (△). After 60 min at 30° the product was analyzed by agarose gel electrophoresis. (*B*) The ATPase reaction mixtures contained 0.15 pmol of relaxed ColE1 DNA, the indicated amounts of [γ-^{32}P]ATP, 4 units of subunit A and 2 units of subunit B, and either no novobiocin (○), 41 nM novobiocin (□), or 160 nM novobiocin (△). After 2 hr at 30° the amount of $^{32}P_i$ was measured by thin layer chromatography.

M for the K_m for ATP.[¶] Although studied less exhaustively, coumermycin A_1 also seems competitive with ATP in the supercoiling and ATPase assays; the calculated K_i was 4×10^{-9} M. App[NH]p, unlike novobiocin and coumermycin A_1, has a structure very similar to that of ATP (20). As shown by the Dixon plots (21) in Fig. 3, it is a potent inhibitor of supercoiling and ATPase activities; both assays gave the same K_i value of 3.4×10^{-6} M. The data indicate a competitive interaction with ATP which was confirmed by a Lineweaver–Burk analysis in a separate experiment measuring ATPase ($K_i = 3.9 \times 10^{-6}$ M). Thus, ATP and App[NH]p probably bind to the same site(s) on DNA gyrase. The average value for the K_i of 3.7×10^{-6} M is two orders of magnitude less than the K_m for ATP of 3.2×10^{-4} M, the mean value from three experiments.

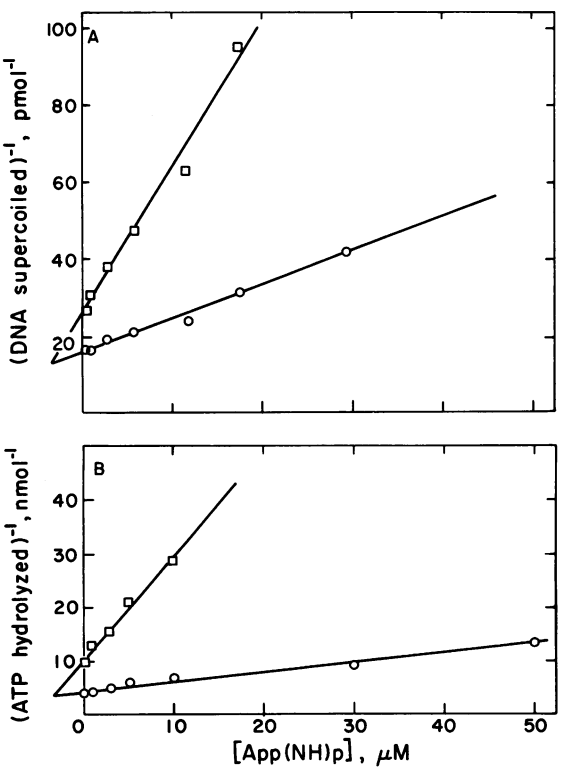

FIG. 3. Inhibition of DNA gyrase by App[NH]p. Supercoiling (*A*) and ATPase (*B*) reaction mixtures contained 0.11 pmol of relaxed ColE1 DNA, the indicated amounts of App[NH]p, 8 units of subunit A and 4 units of subunit B, and either 170 μM ATP (○) or 670 μM ATP (□). Incubation was for 60 min at 30°.

[¶] The kinetic constants are apparent values, because they were obtained with only a single concentration of DNA.

Supercoiling Results from ATP or App[NH]p Binding. The

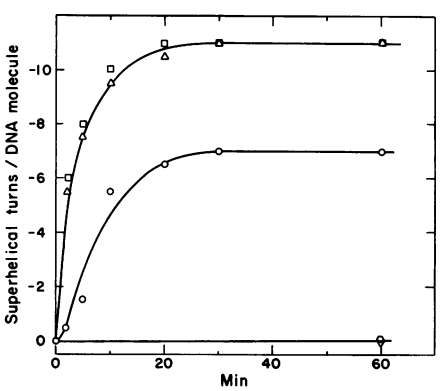

FIG. 4. Time course of App[NH]p-promoted supertwist introduction. The 0.14-ml reaction mixtures contained 47 fmol of relaxed ColE1 DNA and 85 units of subunit A (0.85 pmol of A protomer) plus 110 units of subunit B (○), 170 units of subunit A (1.7 pmol of A protomer) plus 220 units of subunit B (△), 260 units of subunit A (2.6 pmol of A protomer) plus 330 units of subunit B (□), 800 units of subunit A (○), or 240 units of subunit B (▽). After 30 min at 30°, 0.29 mM App[NH]p was added and incubation was continued at 30°. At the times indicated, 17-μl portions of each reaction mixture were withdrawn and mixed with the $NaDodSO_4$ stop solution, and the number and sign of the supertwists in the DNA were determined by the band counting method by using electrophoresis at both 4° and 23°. The relaxed substrate has, by definition, zero supertwists under supercoiling assay conditions. This is close to the absolute value because excess rat liver DNA untwisting enzyme introduced only about +0.5 superhelical turn.

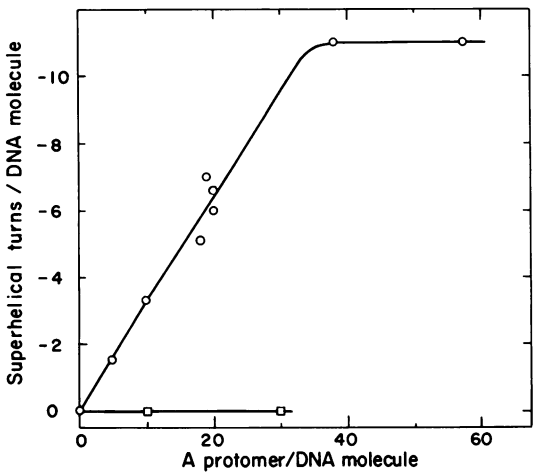

FIG. 5. Extent of supertwisting introduced by App[NH]p as a function of enzyme concentration. The final extent of supercoiling introduced by gyrase reconstituted with a 35% excess of subunit B was measured as in Fig. 4 with (○) or without (□) 0.29 mM App[NH]p.

results thus far seem paradoxical. Binding of ATP or App[NH]p drives at least a portion of the energy transduction cycle as measured by their very similar alteration of the cleavage pattern. Yet hydrolysis of ATP plays a vital role in supercoiling, because App[NH]p is a powerful inhibitor, competitive with ATP. However, DNA gyrase catalyzes supercoiling and ATP hydrolysis (6) but does not turn over in the cleavage reaction, and thus the paradox is resolved if binding of ATP drives one round of supertwisting but hydrolysis is necessary for turnover. A key prediction of this hypothesis was verified. Negative superhelical turns were introduced into closed duplex DNA in the presence of App[NH]p with substrate levels of enzyme (Fig. 4). The extent of supercoiling with App[NH]p was much less than that fueled by ATP and it rapidly reached a plateau value that was dependent on enzyme concentration (Fig. 4 and below). The addition of ATP led, after a lag, to complete supercoiling (data not shown); therefore the enzyme was still active after the reaction had reached a plateau. The App[NH]p-induced supercoiling required both subunits A and B (Fig. 4) and was inhibited by novobiocin (data not shown).

The stoichiometry between App[NH]p-induced supercoils and enzyme molecules was measured with gyrase reconstituted with an excess of subunit B and limiting amounts of subunit A (Fig. 5). The number of superhelical turns per DNA molecule reached a limit in each reaction that was proportional to enzyme concentration until there were about 35 subunit A protomers per ColE1 DNA molecule—this represents about one protomer per 200 base pairs. Before this limit was reached, a -0.32 ± 0.04 turn was introduced per A protomer. The value may be an underestimate because it is not known whether all subunit molecules were enzymatically active and to what extent the B–R activity of gyrase was undoing supercoiling in a Sisyphean manner. In the absence of App[NH]p, no supercoils were introduced, even at high enzyme levels (Fig. 5).

DISCUSSION

The generation of negative superhelical turns by DNA gyrase probably involves a series of reactions including binding, twisting, breakage and reunion of DNA, binding and cleavage of ATP, release of ADP and P_i, and conformational changes of the enzyme. In the face of this complexity, we have studied three reactions involving energy transduction that monitor portions of the complete reaction—ATPase, App[NH]p-induced supercoiling, and ATP- or App[NH]p-driven cleavage site reorganization. All three reactions require both subunits A and B and are inhibited by low concentrations of novobiocin or coumermycin A_1. Each highlights a different aspect of the total reaction sequence. ATPase is a quantitative partial reaction that provided the simplest method for demonstrating the competitive nature of several inhibitors. In the reactions with App[NH]p, the events prior to ATP hydrolysis and enzyme turnover are dissociated from the complete sequence. The movement of the primary cleavage site affords a view of the alteration of enzyme interaction with the DNA that accompanies nucleotide binding. It probably does not involve gyrase release and rebinding, because a large amount of competitor DNA introduced just prior to ATP addition does not affect the cleavage reorientation (unpublished data). The binding of nucleotide could result in translation of the enzyme along the DNA from one cutting site to the other. Alternatively, the enzyme may bind simultaneously to both sites and the presence or absence of nucleotide influences which site is cleaved by altering the conformation of the enzyme.

The mechanism of action of novobiocin and coumermycin A_1 has been elucidated—they prevent the binding of ATP to DNA gyrase. The essential clue was their interference with the translation of the cleavage site induced by ATP or App[NH]p. This identified the sensitive step as one in energy transduction that precedes ATP hydrolysis. Competitive inhibition with respect to ATP in both the supercoiling and the ATPase reactions showed that the step is ATP binding. All effects of the antibiotics on gyrase can now be explained. Activities of gyrase that are not dependent on an energy source are immune to the drugs; this includes B–R, cleavage, and binding of gyrase to DNA (7, 8; unpublished data). Reactions that require energy input, including ATP hydrolysis, supercoiling, and cleavage site reorientation, are extremely sensitive to the drugs and the effect of ATP is cancelled (7, 8). Thus the presence of the drugs and the absence of ATP equivalently expose the B–R activity of gyrase and yield the same cleavage pattern.

Despite the selective and competitive effect of novobiocin and coumermycin A_1 on ATP binding, there is no striking structural homology between ATP and the drugs. Indeed, novobiocin is not known to affect any other ATP-requiring enzyme at these low concentrations. The weak analogy between the structure of the adenine-ribose moiety of ATP and the coumarin-noviose moiety present in both drugs cannot account for their competitive binding. On the one hand, ATP binding is highly specific because related compounds such as GTP, CTP, UTP, dATP, ADP, and AMP do not alter the cleavage pattern or promote appreciable supercoiling. On the other hand, the K_i values for the drugs are very low—more than four orders of magnitude less than the K_m for ATP. (This impressively tight binding was previously underestimated because of millimolar concentrations of the competitor ATP in gyrase assays.) Therefore, novobiocin and coumermycin A_1 may block ATP access without sharing binding sites, or the enzyme conformations competent for binding ATP and the drugs could be incompatible.

App[NH]p, like novobiocin and coumermycin A_1, acts as a potent competitor of ATP in inhibiting catalysis of supercoiling and ATPase (Fig. 3). However, in two reactions employing substrate levels of gyrase, it replaced ATP—it had essentially the same effect as ATP in altering the cleavage pattern (Fig. 1) and it supported limited supercoiling (Figs. 4 and 5). A hypothesis that explains these results is that binding of App[NH]p and ATP leads to one round of supercoiling but that enzyme turnover requires ATP hydrolysis.

The importance of App[NH]p-supported supercoiling for our conclusions dictates validation and careful analysis. First, App[NH]p-promoted supercoiling shared with ATP-fueled supercoiling the requirement for both subunits A and B (Fig.

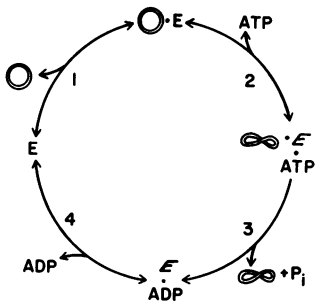

FIG. 6. Scheme for energy transduction by DNA gyrase. The two gyrase conformations are depicted as E and *E* and the DNA substrate is depicted as relaxed closed duplex DNA or a folded figure "8" form.

4) and sensitivity to novobiocin and coumermycin A_1. Secondly, the supercoils reflect a change in the linking number of DNA and are not just maintained by some physical interaction between gyrase and DNA because they survived treatment with $NaDodSO_4$ and proteinase K. Third, the reaction required App[NH]p (Fig. 5); successive purification of the analogue by two distinct methods which resolved it from ATP ensure that this is not due to contamination with ATP. Fourth, the negative supercoiling was an active instead of a passive process, because incubation of the substrate with excess rat liver DNA untwisting enzyme under standard gyrase conditions resulted in no negative supercoiling (data not shown). Fifth, App[NH]p promoted a limited topological change (Fig. 4) that was proportional to gyrase concentration (Fig. 5); about 0.3 negative supertwist was introduced per subunit A protomer. This is consistent with App[NH]p's stopping the supercoiling reaction prior to enzymatic turnover. After the plateau was reached, the enzyme was still active because subsequent addition of ATP resulted in complete supercoiling of the DNA. Slow hydrolysis of App[NH]p has not yet been ruled out directly, but the limited topological change found makes it unlikely that it is important for supercoiling. Of the many ATP-requiring enzymes with which it interacts, only bacterial alkaline phosphatase hydrolyzes the γ phosphate at an appreciable rate (20). Sixth, beyond 30–35 subunit A protomers per ColE1 DNA molecule, no increase in the extent of supercoiling was obtained (Fig. 5). This may represent the physical limit of the ColE1 DNA molecule for binding gyrase. If all the A protomers were bound, the packing ratio would be approximately one subunit A protomer per 200 base pairs.

The features emerging for chemical energy-fueled movement in diverse natural processes, including muscle contraction, tRNA binding to ribosomes, peptidyl tRNA translocation, active transport, oxidative phosphorylation, and photophosphorylation, are remarkably similar to our results on DNA supercoiling (22–25). In general, a nucleoside triphosphate seems to be an allosteric effector for a conformational change that leads to one round of movement; cofactor dissociation is a slow step which is facilitated by hydrolysis of the β-γ anhydride bond and frequently by interaction with another protein or subunit of the same enzyme. For example, a nonhydrolyzable GTP analogue leads to a single translocation of peptidyl tRNA on the ribosome in the presence of substrate levels of G-factor (24). Fig. 6 shows an adaptation to gyrase of the energy transduction scheme used by some workers in these fields, in particular by Hill (23). A specific energy cycle is shown only to demonstrate the plausibility of conformational changes associated with binding of ATP and ADP for catalysis by gyrase. In step 1, gyrase (E) binds to closed duplex DNA in the absence of cofactor. In step 2, ATP (or App[NH]p) binding results in a conformational change in gyrase to *E* form that leads to a movement of the DNA relative to enzyme and, after B–R, to supercoiling.‖ This transition is directly analogous to the nucleotide-induced change in the myosin crossbridge conformation that is postulated to underlie the sliding of filaments during muscle contraction (25). The conformational change comes about because E is generally more stable than *E*, but the order is reversed by ATP; i.e., ATP binds preferably to *E*. In step 3, the hydrolysis of ATP accompanies dissociation of the DNA; this irreversible step provides direction to the cycle. Without dissociation, alternation between the two enzyme conformations would lead merely to oscillation of the DNA, but it does not follow that gyrase is strictly distributive (it is not) because enzyme can still be bound to DNA at another site. The cycle is completed in step 4, in which the release of ADP returns the enzyme to the original conformation, E. Nucleotide release occurs in this step because ADP binds less well than ATP.

‖ Liu and Wang (3) have discussed recently how movement of DNA past gyrase could be involved in supercoiling.

We are indebted to Dr. K. Arai for pointing out the fruitful analogy between energy transduction in DNA supercoiling and protein synthesis that led to the experiments with substrate levels of DNA gyrase. Dr. R. Depew provided valuable advice on the measurement and energetics of supercoiling. This work was supported by National Institutes of Health Grants GM-21397 and CA-19265. N.P.H., P.O.B., and C.L.P. were supported by Training Grants GM-07190, GM-07281, and GM-00780, respectively.

1. Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3872–3876.
2. Marians, K. H., Ikeda, J., Schlagman, S. & Hurwitz, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1965–1968.
3. Liu, L. F. & Wang, J. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2098–2102.
4. Kreuzer, K. N., McEntee, K., Geballe, A. P. & Cozzarelli, N. R. (1978) *Mol. Gen. Genet.*, in press.
5. Cozzarelli, N. R. (1977) *Annu. Rev. Biochem.* **46**, 641–668.
6. Higgins, N. P., Peebles, C. L., Sugino, A. & Cozzarelli, N. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1773–1777.
7. Sugino, A., Peebles, C. L., Kreuzer, K. N. & Cozzarelli, N. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4767–4771.
8. Gellert, M., Mizuuchi, K., O'Dea, M. H., Itoh, T. & Tomizawa, J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4772–4776.
9. Bourguignon, G. J., Levitt, M. & Sternglanz, R. (1973) *Antimicrob. Agents Chemother.* **4**, 479–486.
10. Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 4474–4478.
11. Ryan, M. J. (1976) *Biochemistry* **15**, 3769–3777.
12. Wang, J. C. & Liu, L. F. (1979) in *Molecular Genetics*, ed. Taylor, J. H. (Academic, New York), Part 3, in press.
13. Depew, R. E., Liu, L. F. & Wang, J. C. (1978) *J. Biol. Chem.* **253**, 511–518.
14. Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3800–3804.
15. Shure, M. & Vinograd, J. (1976) *Cell* **8**, 215–226.
16. Keller, W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4876–4880.
17. Kornberg, A., Scott, J. F. & Bertsch, L. L. (1978) *J. Biol. Chem.* **253**, 3298–3304.
18. Yount, R. G., Babcock, D., Ballantine, W. & Ojala, D. (1971) *Biochemistry* **10**, 2484–2489.
19. Lovett, M. A., Katz, L. & Helinski, D. R. (1974) *Nature (London)* **251**, 337–340.
20. Yount, R. G. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol.* **43**, 2–57.
21. Dixon, M. (1953) *Biochem. J.* **55**, 170–171.
22. Boyer, P. D., Hackney, D. D., Choate, G. L. & Janson, C. (1978) in *The Proton and Calcium Pumps*, eds. Azzone, G. F., Kungenberg, M. & Quagliarello, E. (Elsevier, North-Holland Amsterdam), pp. 17–28.
23. Hill, T. L. (1969) *Proc. Natl. Acad. Sci. USA* **64**, 267–274.
24. Inoue-Yokosawa, N., Ichikawa, C. & Kaziro, Y. (1974) *J. Biol. Chem.* **249**, 4321–4323.
25. Taylor, E. W. (1978) *CRC Crit. Rev. Biochem.*, in press.