Deoxyribonucleic Acid Gyrase–Deoxyribonucleic Acid Complex Containing 140 Base Pairs of Deoxyribonucleic Acid and an $\alpha_2\beta_2$ Protein Core†

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ABSTRACT: Staphylococcal nuclease digestion of the complex between DNA and DNA gyrase yields a gyrase–DNA core particle composed of a 140 base pair DNA segment and an active gyrase enzyme. The partial specific volume and $s_{20,w}$ of this purified core complex are measured to be 0.70 cm^3/g and 14.5 S, respectively, by sedimentation measurements in H_2O and D_2O media. The molecular weight of the core complex estimated from equilibrium centrifugation is 470 000; the ratio of the translational frictional coefficient to that of the unsolvated equivalent sphere is calculated to be 1.9. Treatment of free gyrase in solution with dimethyl suberimidate gives three cross-linked species of roughly equal amounts that can be identified as α_2, $\alpha_2\beta$, and $\alpha_2\beta_2$. When the gyrase core complex is treated with the same cross-linking agent, 70–80% of the protein is converted to the $\alpha_2\beta_2$ species. These results establish that the gyrase–DNA core complex contains a 140 base pair DNA segment and a tetrameric $\alpha_2\beta_2$ protein.

DNA gyrase was discovered 4 years ago as an activity in *Escherichia coli* that catalyzes the ATP[1] hydrolysis coupled negative supercoiling of DNA (Gellert et al., 1976a). In vivo studies with specific inhibitors of gyrase indicate that the enzyme is responsible for the maintenance of *E. coli* chromosome, or intracellular phage λ DNA, in an underwound or negatively superhelical state (Gellert et al., 1976b; Kikuchi & Nash, 1978; Drlica & Snyder, 1978; Snyder & Drlica, 1979). A multitude of physiological effects of complete or partial inactivation of gyrase have been reported. These include cessation of DNA replication, reduction of transcription of certain operons, inhibition of DNA repair, curing of plasmids, and impairment of phage λ integrase promoted site-specific recombination [for a review, see Cozzarelli (1980)]. Thus the functional importance of gyrase in prokaryotic organisms is well established.

Two structure genes have been identified for the *E. coli* enzyme. the *gyrA* gene codes for a 105 000 peptide weight subunit and the *gyrB* gene codes a 95 000 peptide weight subunit (Mizuuchi et al., 1978; Higgins et al., 1978). Two readily separable subunits A and B of *Micrococcus luteus*, with peptide weights of 115 000 and 97 000, respectively, have been purified (Liu & Wang, 1978a). These subunits correspond respectively to the *E. coli* proteins of the same designations, as evidenced by studies on reconstituted intergeneric hybrid enzymes (Brown et al., 1979).

The complex between DNA and DNA gyrase resembles the nucleosome structure of the eukaryotic genome in that the DNA appears to be wrapped outside the protein core [Liu & Wang, 1978a,b; for reviews on the nucleosome structure, see Kornberg (1977) and Felsenfeld (1978)]. Similar to the studies on nucleosomes, three lines of evidence support a DNA wrapped around protein model for the gyrase complex. Staphylococcal nuclease digestion of the complex yields a protected DNA fragment ~140 base pairs in length. Digestion with pancreatic DNase I gives, upon denaturation of the DNA, well-defined single-stranded DNA fragments that differ in length approximately in multiples of ten nucleotides. Finally, when the catalytic activity of gyrase is blocked by the omission of ATP or the addition of an inhibitor, it can be readily demonstrated that the linking number of a DNA covalently closed in the presence and absence of gyrase is different.

To gain further information on the structure of the gyrase–DNA complex, we have isolated a core particle from a staphylococcal nuclease digest of the complex. This core complex is shown to be consisted of 140 base pairs of DNA and an active gyrase enzyme. Sedimentation measurements in H_2O and D_2O media give a partial specific volume of 0.70 cm^3/g for this core complex, and the frictional coefficient of the particle relative to that of the unsolvated equivalent sphere is calculated to be 1.9. Protein cross-linking studies reveal a subunit structure of the form $\alpha_2\beta_2$ for the enzyme in solution and bound to DNA in the gyrase core particle.

Materials and Methods

Preparation of M. luteus DNA Gyrase. Two hundred fifty grams of spray dried *M. luteus* cells (Miles Laboratories) were washed in 1.4 L of 0.01 M Tris-HCl, pH 8.0, and resuspended in 4 L of 0.2 M sucrose, 0.02 M Tris-HCl, pH 8.0, 0.01 M 2-mercaptoethanol, and 23 μg/mL phenylmethanesulfonyl fluoride (PMSF). Eighty-five milliliters of lysozyme (1 mg/mL) in 0.01 M Tris-HCl, pH 8.0, was added, and cells were lysed at 37 °C with continuous stirring. After 15 min, 0.2 mL of 1.0 M $MgCl_2$ was added and lysis was allowed to continue for an additional 25 min. Forty milliliters of 1.0 M $MgCl_2$ was then added, and the reaction was quenched with 4 L of ice-cold water. All subsequent steps were performed at 4 °C.

Four hundred milliliters of 10% (w/v) Polymin P (Bethesda Research Laboratories) adjusted to pH 7.9 in 0.01 M Tris-HCl was added dropwise to the cell lysate and stirred continuously for 10 min. The Polymin P pellet was collected by centrifugation at 9000 rpm in a JA10 rotor (Beckman) for 10 min and then blended for 10 min in a 1 gallon Waring blender in 2 L of TBEG buffer [0.05 M Tris-HCl (pH 7.5), 0.01 M 2-mercaptoethanol, 0.1 mM Na_2EDTA, and 5% (w/v) glycerol] plus 0.2 M NaCl. Following centrifugation, the su-

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[1] Abbreviations used: ATP, adenosine 5′-triphosphate; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

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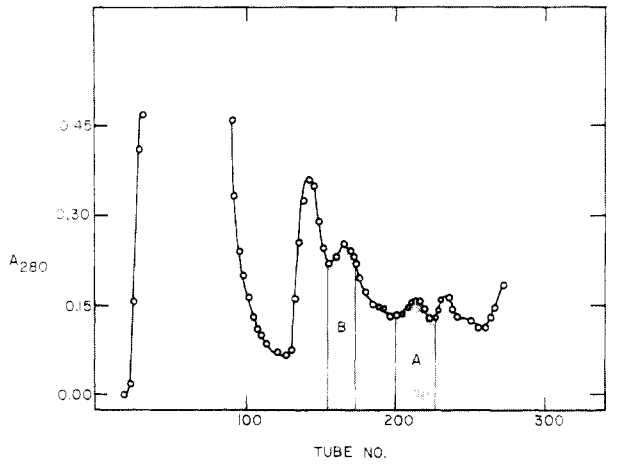

FIGURE 1: Resolution of gyrase A and B subunits by chromatography on hydroxylapatite. Gyrase was eluted from a 3.4 × 20 cm column by a 1 L linear gradient of 0.03–0.25 M potassium phosphate (pH 7) containing 0.01 M 2-mercaptoethanol and 5% (w/v) glycerol. The volume of each fraction was about 6.2 mL, and the flow rate was ~50 mL/h. The gradient was started at tube 96.

pernatant was discarded and the pellet was resuspended in 2 L of 0.5 M NaCl–TBEG buffer by low-speed blending for 10 min. The Polymin P pellet was removed by centrifugation and protein in the supernatant was recovered by ammonium sulfate precipitation [0.35 g of $(NH_4)_2SO_4$/g of solution]. The yellow pellet that resulted was back-extracted with 800 mL of 1.84 M ammonium sulfate in TBEG buffer by low-speed blending for 10 min. Insoluble material was removed by centrifugation. This procedure was repeated successively with 400 mL solutions of 1.74, 1.48, and 1.33 M ammonium sulfate in TBEG buffer. Nearly all of the ATP-dependent DNA supercoiling activity was contained in the 1.48 M ammonium sulfate fraction, and protein was recovered from this solution by a second ammonium sulfate precipitation.

The crude gyrase preparation was dissolved in 40 mL of 0.03 M potassium phosphate (pH 7), 0.01 M 2-mercaptoethanol, and 5% (w/v) glycerol, dialyzed against the same buffer for 1.5 h, and centrifuged for 10 min at 12 000 rpm in a JA20 rotor. The supernatant was loaded on a 5 × 95 cm Bio-Gel A1.5M (Bio-Rad, 100–200 mesh) column. Gyrase activity was eluted between 0.4 and 0.54 column volume. The pooled activity was loaded on a 3.4 × 20 cm hydroxylapatite (Bio-Rad) column equilibrated with the same buffer and eluted with a 1 L linear gradient of 0.03 M–0.25 M potassium phosphate (pH 7) containing 0.01 M 2-mercaptoethanol and 5% (w/v) glycerol. At this stage the two gyrase subunits separated, the B subunit eluting between 0.07 and 0.12 M potassium phosphate and the A subunit eluting between 0.13 and 0.18 M potassium phosphate (Figure 1). No DNA supercoiling activity was detectable in fractions in the shaded areas shown in the figure unless a fraction from the shaded area labeled A is mixed with a fraction from the shaded area labeled B, as first reported by Liu & Wang (1978a).

Crude gyrase A and B subunits from pooled fractions of each of the shaded areas shown in the figure represent approximately 5–10% of the total 280-nm absorbance of the material loaded on the column. These were concentrated by ammonium sulfate precipitation (0.35 g/g), redissolved in 10 mL of the low-salt hydroxylapatite column buffer plus 0.1 mM EDTA, and dialyzed against that buffer. Crude subunit A protein was fractionated in a 2 × 16.5 cm P-11 phosphocellulose column employing a linear 0.03–0.25 M potassium phosphate gradient to yield 4.6 mg of purified protein. The crude subunit B protein was purified by passage through a 2 × 11 cm P-11 column equilibrated with the dialysis buffer. Approximately 30–40% of the total protein was retained by the column and was discarded. The fraction that passed through was dialyzed against 0.1 M KCl–TBEG buffer and loaded on a 3 × 8 cm DE-52 column. The B protein was eluted by a linear gradient of 0.1–0.4 M KCl in TBEG and pooled into fraction BI (1.3 mg) and the less pure sample BII (1.2 mg). The three gyrase subunit fractions (fractions A, BI, and BII) were concentrated by ammonium sulfate precipitation and dialyzed overnight into a storage buffer of 0.1 M Tris-HCl (pH 7.5), 0.02 M 2-mercaptoethanol, 0.5 mM Na_2EDTA, and 50% (w/v) glycerol.

Purification of gyrase to homogeneity was achieved by stepwise elution of the reconstituted holoenzyme on double-stranded DNA cellulose. Gyrase subunit B (fraction BII, 0.8 mg) was combined with an equal weight of subunit A (fraction A), diluted 10-fold with a column buffer [0.035 M Tris-HCl (pH 7.5), 0.02 M Mg^{2+}, 0.02 M 2-mercaptoethanol, 1.0 mM spermidine, and 20% (w/v) glycerol], and incubated at 30 °C for 10 min. The reconstituted enzyme was loaded on a 1.5 × 3 cm (5.3 mL) DNA–cellulose column equilibrated with the column buffer given above. The contaminating proteins washed through the column and gyrase was eluted with column buffer containing 0.2 M ammonium sulfate. The enzyme was dialyzed into storage buffer as described previously.

Preparation of Gyrase–DNA Complex. T7 DNA was extracted from phage that had been banded in CsCl and dialyzed into 0.1 M Tris-HCl (pH 7.5)–0.5 M NaCl–0.025 M EDTA. The phage suspension was extracted 5 times with distilled phenol until a clear water–phenol interface was obtained. The aqueous phase was then extracted 3 times with water-saturated ether, and the DNA was ethanol precipitated and dialyzed into 0.02 M Tris-HCl (pH 7.5)–0.02 M NaCl–0.5 mM EDTA.

Gyrase was reconstituted onto T7 DNA at a ratio of 1 protein tetramer/200 DNA base pairs. The reaction mixture consisting of 400 μL of 0.077 M Tris-HCl (pH 7.8), 0.015 M 2-mercaptoethanol, 0.02 M Mg^{2+}, 1.0 mM Ca^{2+}, 0.4 mM EDTA, 50 μg/mL bovine plasma albumin, 38% glycerol, 125 μg/mL DNA, 210 μg/mL gyrase subunit A, and 190 μg/mL gyrase subunit B was incubated at 30 °C for 20 min, and 40 μL of a 10 units/μL stock of staphylococcal nuclease (Worthington Biochemical Corp.) was added. Digestion was allowed to proceed for 2.5 min, and the reaction was then quenched with 30 μL of 0.1 M Na_3EGTA.

Cross-Linking with Dimethyl Suberimidate. Gyrase samples to be cross-linked were dialyzed overnight into buffer C [0.01 M triethanolamine (pH 8.5), 0.02 M Mg^{2+}, 0.02 M 2-mercaptoethanol, and 10% glycerol] and diluted to give a final protein concentration of 20–120 μg/mL. Twenty milligrams of dimethyl suberimidate (Pierce Chemical Co.) was dissolved in 1 mL of buffer C, and the pH was adjusted to between 9 and 9.5 with 1 N NaOH. One volume of the suberimidate solution was added to 4 volumes of DNA to give a final concentration of 4 mg/mL in dimethyl suberimidate. Cross-linking was allowed to proceed for 0.5–1 h at 30 °C, and the reaction was quenched with one-fifth of the total volume of 1.0 M ethanolamine (pH 8). Gyrase–DNA complex purified after staphylococcal nuclease digestion was cross-linked at an A_{260} of 0.1 in buffer C containing 1 mM spermidine and 20% instead of 10% glycerol. Gyrase cross-linked in this buffer gave identical results with those obtained in buffer C. Monomeric proteins were cross-linked with dimethyl suberimidate by the procedure of Carpenter & Harrington (1972). Fifty microliters of a solution containing 5 mg of protein and 6 mg of dimethyl suberimidate in 1.0 mL of 0.2

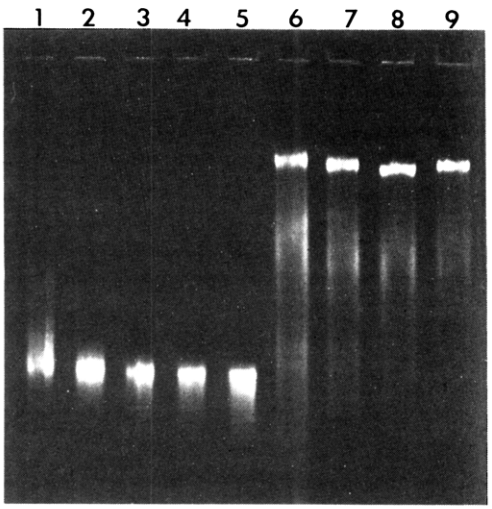

FIGURE 2: Time course of staphylococcal nuclease digestion of T7 DNA–*M. luteus* DNA gyrase complex. 40-μL aliquots of a 400-μL digest were removed at time intervals and analyzed by electrophoresis on 5% polyacrylamide–TBM gels. Lanes 1–5 contain protein-free DNA corresponding to digestion times 2, 4, 6, 8, and 10 min. Lanes 6–9 contain the gyrase–DNA complex after digesting for 2, 4, 6, and 8 min.

M triethanolamine (adjusted to pH 8.5) was combined with 50 μL of a second solution composed of 2% sodium dodecyl sulfate, 2% 2-mercaptoethanol, and 0.2 M triethanolamine (pH 8.5), and the mixture was frozen at −20 °C for 19 h. Following 2 h of incubation at 37 °C, the reaction was quenched by the addition of 0.15 volume of 1.0 M ethanolamine.

Analytical Procedures. Polyacrylamide gel electrophoresis of the staphylococcal nuclease digested gyrase–DNA complex and DNA was performed as described by Maniatis et al. (1975) with 5% slab gels in Tris–boric acid–magnesium (TBM) buffer. Gels (16 × 20 × 0.3 cm) were run at 100 V for 2–6 h, stained with 2 μg/mL ethidium bromide, and photographed on a UV light box. Proteins were analyzed on 3.5% polyacrylamide gels containing 0.075 M potassium phosphate (pH 7) and 0.1% sodium dodecyl sulfate. Gels (16 × 20 × 0.1 cm) were run at 90 V for 6–8 h and stained with Coomassie blue. Gyrase supercoiling assays were performed as described earlier (Liu & Wang, 1978b).

Analytical sedimentation was performed in a Spinco Model E ultracentrifuge equipped with a photoelectric scanning system. Scans were taken at 265 nm of solutions with an absorbance of 0.25–0.35 at 260 nm. Heavy water (Bio-Rad, 99.8% deuterated) was used for density enhancement in the differential sedimentation experiments. Density was measured relative to water at 4 °C by direct weighing of a 100-μL calibrated micropipet. Relative viscosity, η/η_0, was determined with an Ostwald viscometer in a constant-temperature reservoir at 4 °C. Protein determination was performed as described by Bradford (1976), using high-purity bovine plasma albumin as a standard. Double-stranded DNA–cellulose was either purchased from P-L Biochemicals or prepared by the procedure of Alberts & Herrick (1971).

Results

Isolation of a Gyrase–DNA Core Complex. Figure 2 depicts the electrophoretic patterns of the complex between gyrase and phage T7 DNA after digestion with staphylococcal nuclease for various times. For the particular gel electrophoresis system used (5% polyacrylamide, Tris–borate–Mg^{2+} buffer), intact T7 DNA hardly enters the gel. Upon digestion of the complex with the nuclease and then Pronase, a sharp band is seen (lanes 1–5). With a restriction endonuclease

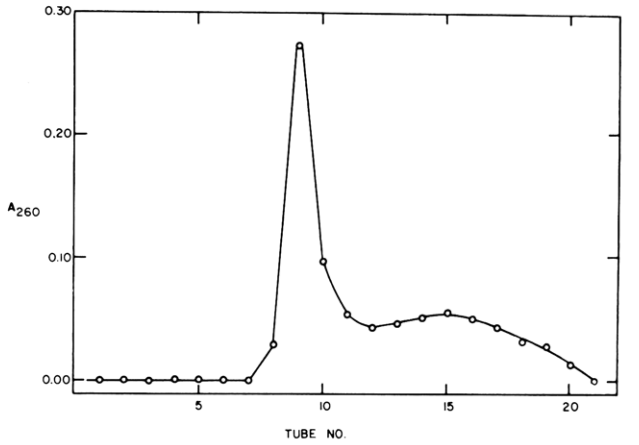

FIGURE 3: Gel filtration of gyrase core particles on Bio-Gel A1.5M (100–200 M). 400 μL of a staphylococcal nuclease digest of the gyrase–DNA complex was loaded on a 1.0 × 26 cm column and eluted with a column buffer containing 0.035 M Tris-HCl (pH 7.5), 0.02 M $MgCl_2$, 0.02 M 2-mercaptoethanol, 1 mM spermidine, 1 mM EGTA, and 20% glycerol. The volume of each fraction was about 1 mL.

*Hae*III digest of phage PM2 DNA as calibration markers (Kovacic & van Holde, 1977), the mobility of this band corresponds to that of a DNA fragment with 143 ± 3 base pairs, in agreement with the result of Liu & Wang (1978b). In contrast to staphylococcal nuclease digestion of chromatin, no higher molecular weight bands corresponding to dimeric or oligomeric particles are observed. There are also no distinct lower molecular weight bands resulting from cleavage at unique sites within the 140 base pairs.

If the Pronase digestion step is omitted and the staphylococcal nuclease digested sample is loaded on the gel directly after the addition of EGTA to stop the reaction, a slower migrating band stainable by ethidium results (lanes 6–9). Electrophoresis of the material recovered from the band on a polyacrylamide–dodecyl sulfate gel reveals the presence of both subunits A and B of gyrase. This suggests that the staphylococcal nuclease resistant core complex between DNA and gyrase is stable during electrophoresis. Replacement of the Tris–borate–Mg^{2+} electrophoresis buffer with Tris–borate–EDTA buffer, however, apparently results in the dissociation of the complex, and the ethidium-stained fluorescent band now migrates at the faster mobility whether the sample had been treated with Pronase or not.

Purification of the gyrase–DNA core complex was achieved by passage of the staphylococcal nuclease digested sample through a Bio-Gel A1.5M column. As depicted in Figure 3, a sharp peak that elutes in the column void volume is separated from a broad trailing band. Electrophoresis of fractions under the sharp peak, using the Tris–borate–Mg^{2+} buffer system, shows that these fractions contain the core complex (Figure 4). The broad trailing band contains DNA fragments shorter than 140 base pairs.

The Core Complex Contains Equal Moles of Gyrase Subunits A and B and No Other Proteins. Electrophoresis in a polyacrylamide–dodecyl sulfate gel reveals that the purity of our gyrase subunit A preparation is ~90%, with two minor impurity bands (Figure 5a). The larger of the impurity bands has a peptide weight of 78 000. The gyrase subunit B preparation, fraction BI, is ~80% pure and contains about 15–20% of the protein in the 78 000 impurity band (Figure 5b). The peptide weights of the A and B subunits are calculated to be 115 000 and 93 000, respectively, from their mobilities, in reasonable agreement with the previously reported values (Liu & Wang, 1978a). The impurity bands can be removed by

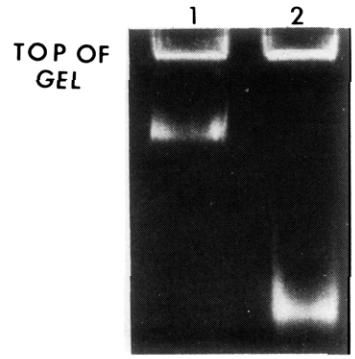

FIGURE 4: 5% polyacrylamide–TBM gel electrophoresis of purified core particles (lane 1) and the same sample treated with sodium dodecyl sulfate (lane 2). The protein-free DNA migrates as a 143 ± 3 base pairs long fragment when compared to a restriction endonuclease *Hae*III digest of PM2 DNA (not shown).

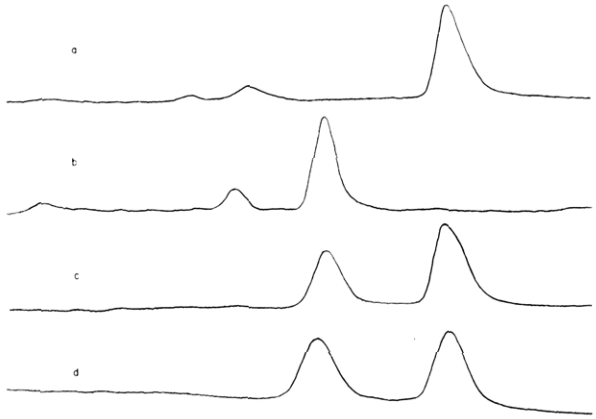

FIGURE 5: Densitometer tracings of DNA gyrase analyzed on 3.5% polyacrylamide–dodecyl sulfate gels. (a) Purified gyrase subunit A, 10 μg; (b) purified gyrase subunit B, fraction BI, 10 μg; (c) gyrase reconstituted with an excess of subunit A after purification on a DNA–cellulose column, 10 μg; (d) protein complement of isolated gyrase–DNA core complex, ~20 μg.

Table I: Sedimentation Measurements of the Gyrase–DNA Core Complex[a]

parameter	expt 1	expt 2
η_1/η_0	1.89	1.87
η_2/η_0	2.29	2.35
ρ_1 (g/cm^3)	1.050	1.054
ρ_2 (g/cm^3)	1.136	1.141
s_1 (S)	4.28	4.37
s_2 (S)	2.91	2.80
$s_{20,w}$ (S)	14.4	14.6
$\bar{v}$ (cm^3/g)	0.69	0.70
M_r	470 000	480 000
f/f_0	1.9	1.8

[a] All measurements were performed at 4 °C. η_0, η_1, and η_2 are the viscosities of pure H_2O and the H_2O and D_2O media used in the measurement; ρ_1 and ρ_2 are the densities of the H_2O and D_2O media, respectively. The molecular weight is calculated from equilibrium sedimentation as described under Materials and Methods. f/f_0 is the ratio of the translational frictional coefficient of the core particle to that of an unhydrated sphere of the same volume.

reconstitution of the purified subunits and chromatography on DNA–cellulose (Figure 5c and Figure 6B, lane 3). The gyrase so obtained is electrophoretically pure and is fully active.

When the core complex isolated from the peak excluded by the Bio-Gel 1.5M column is examined by polyacrylamide–dodecyl sulfate gel electrophoresis, again only the subunits A and B bands are seen. Based on the assumption that the Coomassie blue stain of a band is proportional to the protein

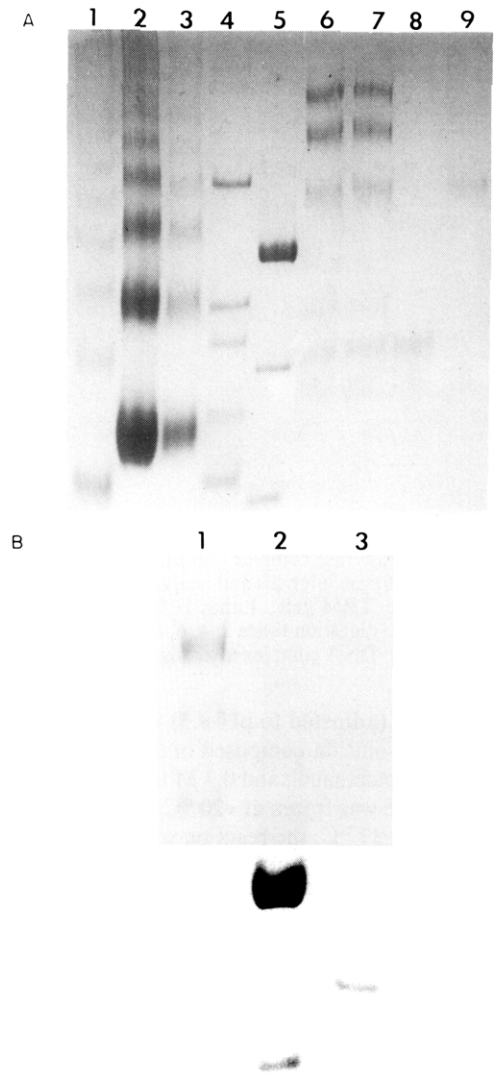

FIGURE 6: (A) 3.5% polyacrylamide–dodecyl sulfate gel electrophoresis of gyrase and other proteins cross-linked with dimethyl suberimidate. (1) Cross-linked ovalbumin, 50 μg; (2) cross-linked bovine serum albumin, 100 μg; (3) cross-linked bovine serum albumin, 20 μg; (4) protein standards (top to bottom)—myosin, galactosidase, phosphorylase, BSA, and ovalbumin; (5) *E. coli* RNA polymerase; (6) cross-linked gyrase (1 h), 15 μg; (7) cross-linked gyrase (0.5 h), 15 μg; (8) cross-linked gyrase subunit B, 10 μg; (9) cross-linked gyrase subunit A, 10 μg. (B) Cross-linking of gyrase–DNA core particles with dimethyl suberimidate. (1) 800 μL of the core complex at 0.1 A_{260}/mL, cross-linked for 1 h; (2) *E. coli* RNA polymerase; (3) free gyrase, 10 μg.

mass, the two subunits appear to be present in equal molar amounts in the core complex, and no other protein is detectable. When the purified core complex is incubated with relaxed PM2 DNA in the presence of ATP, supercoiling of the PM2 DNA results. The supercoiling activity of the core complex is comparable to that of purified gyrase under our assay conditions (results not shown).

Characterization of the Core Complex. Table I summarizes data obtained by sedimenting the core complex at 4 °C in 0.035 M Tris-HCl (pH 7.5), 0.02 M $MgCl_2$, 0.02 M 2-mercaptoethanol, 1 mM spermidine, 1 mM EGTA, and 20% (w/v) glycerol and in the same buffer containing 74% (v/v) D_2O. The sedimentation coefficients in the H_2O and D_2O medium are measured to be 4.28 and 2.91 S, respectively. The partial specific volume $\bar{v}$ of the core complex is calculated from

the procedures of Martin et al. (1956) and Edelstein & Schachman (1976, 1973). The mass ratio k of the complex in 74% D_2O is estimated to be 1.011 by assuming a k value of 1.015 for the protein components in pure D_2O (Martin et al., 1959) and six exchangeable protons per DNA base pair. In this calculation each core particle is assumed to contain two protomers each of the subunits A and B and 140 base pairs. The validity of this assumption will become apparent later. The $\bar{v}$ value is calculated to be 0.70 cm^3/g which corresponds to a value of 0.74 cm^3/g for the partial specific volume of gyrase, assuming additivity of molar volumes of protein and DNA. By use of the $\bar{v}$ value for the complex, a standard two component correction of the sedimentation coefficient in the H_2O medium gives an $s_{20,w}$ of 14.5 S.

The molecular weight of the core particle is estimated by equilibrium centrifugation at 4 °C in the H_2O medium used in the sedimentation coefficient measurements. The slope of a plot of the natural logarithm of the concentration vs. the square of the distance from the center of rotation is 2.16 cm^{-2}, from which the two-component approximation yields a molecular weight of 470 000.

The Core Complex Contains Two Protomers of Each of the Gyrase Subunits. The evidence presented in the preceding sections shows that each core particle contains 143 ± 3 base pairs of DNA and equal molar amounts of gyrase subunits A and B. The molecular weight obtained from equilibrium sedimentation therefore suggests that in each core particle there are two protomers of each of the subunits, i.e., the core particle is of an $\alpha_2\beta_2$ protein structure with 143 base pairs of DNA. The calculated molecular weight for such a particle is 2(115000 + 93000) + 143(640), or 507 000. The number 640 is taken as the average molecular weight of a base pair with Mg^{2+} as the counterion.

Furthermore, when the total protein concentration of the isolated core particles is determined as described under Materials and Methods and the DNA concentration is estimated from the UV absorption spectrum and corrected for the presence of free DNA, the protein to DNA mass ratio is calculated to be 4.2. This is in reasonable agreement with the expected value of 4.5 for an $\alpha_2\beta_2$ protein structure plus 143 base pairs of DNA.

Confirmation of such a structure is obtained by protein cross-linking studies with dimethyl suberimidate (Davies & Stark, 1970). The results are shown in Figure 6. Lanes 1–3 in Figure 6A are electrophoretic patterns of ovalbumin and bovine serum albumin cross-linked by the diimido ester according to the procedure of Carpenter & Harrington (1972). A ladder of well-defined bands appears in each case, indicating the cross-linking of the monomers into oligomers. These oligomers serve as molecular weight markers for identifying the cross-linked gyrase protomers. Additional molecular weight markers were run in lanes 4 and 5. For gyrase subunit A, treatment with the diimido ester yields rapidly an α_2 dimer band with a molecular weight of 230 000 (lane 9). In the presence of spermidine, a second weaker band with a molecular weight of 440 000 is seen (result not shown). Gyrase subunit B by itself gives no cross-linked oligomers with suberimidate (lane 8).

When reconstituted gyrase containing both A and B subunits is treated with suberimidate, three bands with molecular weights 230 000, 330 000, and 420 000 are observed. These values are compared with the calculated molecular weights of various combinations $\alpha_i\beta_j$ of the protomers (Table II). The internally consistent assignments of these three bands as α_2, $\alpha_2\beta$, and $\alpha_2\beta_2$ agree best with the expected molecular weights.

Table II: Identification of Cross-Linked Gyrase Promoters

band	proposed subunit structure	calcd M_r	obsd M_r[a]
	B_2	186 000	
	AB	208 000	
I	A_2	230 000	230 000; 233 000
	B_3	279 000	
	B_2A	301 000	
II	A_2B	323 000	325 000; 333 000
	A_3	345 000	
	B_3A	394 000	
III	A_2B_2	416 000	422 000; 420 000
	A_3B	438 000	

[a] The two values are obtained from separate cross-linking experiments using two different gyrase preparations.

In agreement with the observation that no cross-linking of subunit B is observed in the absence of A, the observed bands are the cross-linking products expected from the covalent linkage of zero, one, and two β protomers to an α_2 dimer.

The pattern of cross-linking of the gyrase–DNA core complex by suberimidate is shown in Figure 6B. Again three oligomeric bands are observed. The mobilities of these bands are identical with those of the corresponding bands resulting from treatment of the gyrase holoenzyme with the cross-linking agent (Figure 6B, lane 1, and Figure 6A, lane 6). For the core particle, 70–80% (by mass) of the protomers are found in the cross-linked $\alpha_2\beta_2$ band. For the free enzyme, the α_2, $\alpha_2\beta$, and $\alpha_2\beta_2$ bands are of roughly equal intensity.

These results support strongly that the gyrase–DNA core complex is of an $\alpha_2\beta_2$ structure containing 140 base pairs of DNA.

Discussion

It is known that under certain conditions, cleavage of DNA by gyrase can occur in a double-stranded manner (Gellert et al., 1977; Sugino et al., 1977; Morrison & Cozzarelli, 1979), resulting in the covalent linkage of a subunit A protomer to each of the two 5′ ends generated (Tse et al., 1980). This suggests that there are two A protomers in each gyrase holoenzyme bound to the DNA. The results we have presented in this paper demonstrate clearly that gyrase binds as an $\alpha_2\beta_2$ tetrameric structure. For the free protein in solution, the suberimidate cross-linking results indicate that the major species of the subunit A by itself is a dimer, in agreement with the data of Sugino et al. (1977). When both subunits A and B are present, at least some $\alpha_2\beta_2$ tetramers are present at the gyrase concentrations used in our protein cross-linking experiments. We have not observed cross-linked protomers of the B subunit. Either the B subunit by itself exists as a monomer or it exists as a multimeric structure but there are no properly located active lysine residues for cross-linking by the diimido ester. For example, dimethyl suberimidate has been shown to exhibit a low reactivity and cross-linking efficiency with the α subunit of RNA polymerase (Hillel & Wu, 1977) and the α protomer of tryptophan synthetase (Davies & Stark, 1970). The gyrase–DNA core complex contains no proteins other than the two subunits A and B. Although an 80 000-dalton contaminant was present in the A and B subunits purified separately, the reconstituted holoenzyme purified by chromatography on DNA–cellulose also contains no detectable contaminant. The gyrase–DNA core complex is stable for at least several days at 4 °C in 0.035 M Tris-HCl (pH 7.5), 0.02 M $MgCl_2$, 0.02 M 2-mercaptoethanol, 1 mM spermidine, 1 mM EGTA, and 20% glycerol. Magnesium ions appear to help the stabilization of the particle, as indicated by the dis-

sociation of the particle during gel electrophoresis in the absence of the divalent ion. Omission of spermidine and the thiol reagent from the buffer also reduces the stability of the particle, and some dissociation of the complex during the gel filtration step occurs as a result. ATP and K^+, although required for the supercoiling activity (Gellert et al., 1976a; Liu & Wang, 1978a), have no apparent effect on the stability of the particles if added in millimolar quantities.

The frictional coefficient of the gyrase–DNA core complex is consistent with its being a globular structure. The ratio of the frictional coefficient to that of the unhydrated equivalent sphere, 1.9, can be compared with the ratio 2.1 for the particle between the arginine-rich histone tetramer $(H3/H4)_2$ and a 140 base pair DNA fragment and the ratio 1.54 for the nucleosome core particle (Klevan et al., 1978; Simon et al., 1978). The $(H3/H4)_2$ and DNA complex has been modeled hydrodynamically as a cylinder of dimensions 450 × 80 × 80 Å (Klevan et al., 1978) and the dimensions of the nucleosome are best described by a cylinder with a diameter of 110 Å and a height of 55 Å (Finch et al., 1977; Crothers et al., 1978). The uncertainty in the hydration term, however, makes it difficult to model the dimensions of the gyrase–DNA core complex from its frictional coefficient.

One of the most interesting aspects of gyrase is its coupling of the energetically favorable process of ATP hydrolysis and the energetically unfavorable process of DNA supercoiling. Conformational changes of the gyrase–DNA complex accompanying ATP binding and hydrolysis undoubtedly occur during catalysis. The isolation of a stable core complex provides a structure for studies on such conformational changes by hydrodynamic and spectroscopic means. We believe that such studies will provide important information on the mechanism of DNA supercoiling by gyrase.

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