

Characterizing Pressure Effects on Winding of the DNA Double Helix

Guo-Qing Tang[#] and Shigeru Kunugi*

Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585

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In order to characterize pressure effects on DNA topological conformations in terms of supercoiling, DNA topoisomerase- and ligase-induced supercoiling assays were applied to analyze several DNA samples from different sources (DNA of bacteriophage ϕX174 RFI, plasmid pBR322, and pUC19). Pressure generally enhanced winding of the DNA double helix. The results were analyzed with respect to the linking number, the rotation angle, and the torsional energy factor (K). K describes the energy cost of increasing DNA linking by one unit; it appeared to decrease linearly with pressure elevations up to at least 180 MPa, which suggested a volume shrinkage with DNA supercoiling.

The involvement of DNA supercoiling in critical cellular processes such as gene replication, transcription, and recombination has been extensively studied.[1] Closed-circular DNA duplexes in the cell are negatively supercoiled and the magnitude of DNA supercoiling is predominantly controlled by a unique class of topoisomerases. The extent of DNA supercoiling is also regulated by numerous other factors (e.g., proteins and small chemical substances) through binding-induced alterations in the DNA structure or through interactions with topoisomerases to affect their activities.[2–4] The topological conformation of DNA (supercoiling) also changes in response to perturbations in environmental conditions (e.g., changes in temperature, ionic concentration, or osmotic stress).[5–15]

Increasing the temperature,[5,6,10,11] lowering the ionic strength,[7,11,13,14] or reducing the water activity[8] have all been reported to induce unwinding of the DNA double helix in vitro. Experimental and theoretical studies have indicated that changes in DNA supercoiling may be associated with alterations in the physical properties of supercoiled DNA, e.g., changes in the persistent length and/or effective diameter, and in the secondary structure (for review see Ref. 15).

Recently we provided the first evidence of in vitro pressure-induced winding of plasmid DNA helix by gel migration analysis of topoisomerase I-catalyzed unwinding of pBR322 DNA.[16] The physiological implications of this finding were verified by analysis of effects at various pressures within the natural range on the earth (below 120 MPa). The pressure range applied here is significantly lower than the high level (e.g., 600 MPa) previously used to observe structural transitions of oligonucleotide duplexes.[17,18] The finding of in vitro pressure-induced increase in plasmid DNA supercoiling suggests that the flexibility of DNA high-order structure should be taken into account when considering the effects of pressure on various DNA-related cellular processes.

Our approach to examining the effects of elevated pressure on DNA supercoiling was based on measurement of the equilibrium distribution of DNA linking numbers determined from supercoiling assays catalyzed by topoisomerase I or T4 DNA ligase.[5,6,16,19] Wang and Vinograd first determined the distribution of DNA topoisomers and measured the free energy of supercoiling.[5,6] A series of DNA topoisomers differing by one Lk unit can be resolved by measuring their electrophoretic mobility in agarose gel.[19] The production of a number of DNA topoisomers reflects the effects of thermal fluctuations in the conformation of DNA molecules during the closure of nick.[5] The method has been widely applied to investigate responses of superhelical conformations of a number of DNA molecules to various environments or exposure to chemical reagents and proteins.

In this work, we studied the topoisomer distributions of several DNA molecules in light of the hydrostatic pressure that is applied to supercoiling assays with topoisomerase I or T4 DNA ligase. It was found that DNA supercoiling was progressively increased while the torsional free energy factor K appeared to be decreased under elevated pressure. Origins for the plasticity of the high-order structure of DNA under pressure were discussed.

Materials and Methods

Enzymes, DNA and Chemicals. Wheat germ DNA topoisomerase I was purchased from Promega Japan (Tokyo). T4 DNA ligase was purchased from Life Technol. Oriental (Tokyo). The highly negatively supercoiled DNA of pBR322, pUC19, and ϕX174RFI were purchased from Boehringer Manheim (Germany), Takara Shuzo (Ohtsu), and Life Tech. Oriental, respectively. Singly nicked DNA was obtained by limited digestion of closed-circle DNA with DNase I (Life Tech.) (2.5×10^{-6} unit/μL) at 14 °C. Linear DNA was obtained by complete digestion of closed-circular DNA molecules by selected type II endonucleases at a single site. Nicked/linear DNA was purified by extraction with phenol/chloroform followed by ethanol precipitation. Crystallized phenol was obtained from Wako Chemical Co. (Osaka) and was equilibrated with 10 mM (M≡mol dm^{-3}) 2-amino-2-(hydroxymethyl)-1,3-

Present address: Department of Molecular Biology, The Scripps Research Institute, 10550, N. Torrey Pines Rd., La Jolla, CA 92117, USA.

propanediol hydrochloride (Tris-HCl) (pH 8.0). The heavy mineral oil used (Sigma; St. Louis, MO, USA) was free of proteases and nucleases. Other chemicals were obtained from Wako Chemical Co. or Nacalai Tesque (Kyoto).

DNA Relaxation Assay Using Topoisomerase I. The standard relaxation assay was conducted in 30 µL of 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, and 10 mM $MgCl_2$, and 5% glycerol. Equilibrium distributions of topoisomers were obtained by incubation of supercoiled/nicked DNA (0.4 µg) with wheat germ topoisomerase I (5 U) at 20 °C and under defined hydrostatic pressures for 24 h. The relaxation reaction was stopped by phenol/chloroform extraction. DNA was recovered by precipitation with 70% cool ethanol and re-suspended in a gel loading buffer {10 mM Tris-HCl, pH 8.0, 2 mM [ethylenedinitrilo]tetraacetic acid (EDTA), 50% glycerol and 0.02% 3′,3″,5′5″-tetrabromophenolsulfonephthalein (bromophenol blue)}.

Ligation of Nicked/Lineralized DNA. The type II restriction endonucleases used to generate cohesive ends of linear DNA of pBR322, pUC119, and ϕX174 RFI were EcoRI (Toyobo, Osaka), Hind III (New England Biolabs., Beverly, MA, USA), and Pst I (Life Tech. Oriental), respectively. Purified nicked or linear DNA was resuspended in 10 mM Tris-HCl (pH 8.0, 0.1 mM EDTA). The ligation reaction mediated by T4 DNA ligase (1 weiss unit) which required Mg^{2+} was conducted in 30 µL of buffer (50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 1 mM adenosine 5′-triphosphate (ATP), 1 mM 1,4-dimercapto-2,3-butanediol (DTT) and 5% polyethylene glycol (average molecular weight = 8000). The reaction mixture was incubated at 20 °C under defined pressures for 20–24 h. Reactions were terminated by the addition of concentrated EDTA to a final concentration of 20 mM. Topoisomer products were purified by phenol/chloroform extraction and ethanol precipitation.

One and Two-Dimensional Electrophoretic Analysis of Topoisomers. Topoisomer products were separated by one and two-dimensional electrophoresis with 0.8 or 1% agarose gel. The buffer for the first dimension was TBE (89 mM Tris-borate, pH 8.1, 1 mM EDTA). Electrophoresis was run at 2–3 V/cm and at 21 ± 1 °C for 12–18 h. After electrophoresis, agarose gels were stained with 0.5 µg/mL of 2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide (ethidium bromide) for 2 h and destained for another 30 min prior to being photographed. In order to differentiate positive and negative topoisomer products unambiguously, two-dimensional electrophoresis was also applied. The TBE buffer used for the second dimension contained 3 µg/mL chloroquine. After the run in the first dimension each agarose gel was rotated by 90° and incubated with 3 µg/mL (7-chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine (chloroquine) in TBE buffer prior to electrophoresis in the second dimension. After electrophoresis, gels containing chloroquine were washed with purified water then stained with ethidium bromide. Agarose gels were photographed under short wave UV-illumination using a Polaroid 667. Photos were scanned and analyzed using NIH Image 1.61 software.

High Pressure Incubations. Manipulations under high pressure have been described elsewhere.[20] A high-pressure apparatus made by Yamamoto Suiatsu Co. (Toyonaka) was used, in which the pressure was raised by electric oil pump through intensifier/separator. The pressure medium in the reaction vessel was water. The temperature in the pressure vessel, where the polyethylene tube (sterilized) containing the reaction mixture was positioned, could be controlled and monitored in situ. The top portion of the test tube was filled with heavy mineral oil to remove air.

Analysis of Data. The model of Wang and Vinograd was used to quantify pressure effects on DNA supercoiling in terms of alterations in the linking numbers.[5,6] The distribution of topoisomers around the major species can be conveniently represented by a Gaussian distribution as

$$C_i/C_m = A \exp [- iK(i + 2\omega_T)/RT] \quad (1),$$

where i is the linking number difference of a topoisomer with the major topoisomer (including sign), C_i and C_m are the amounts of the ith and major topoisomers, respectively. A is a coefficient, and ω_T is the difference between the major peak position and the center of Gaussian distribution (described in terms of turns), originating from the winding of the twist angle upon the ligation reaction. K is a torsional free energy factor

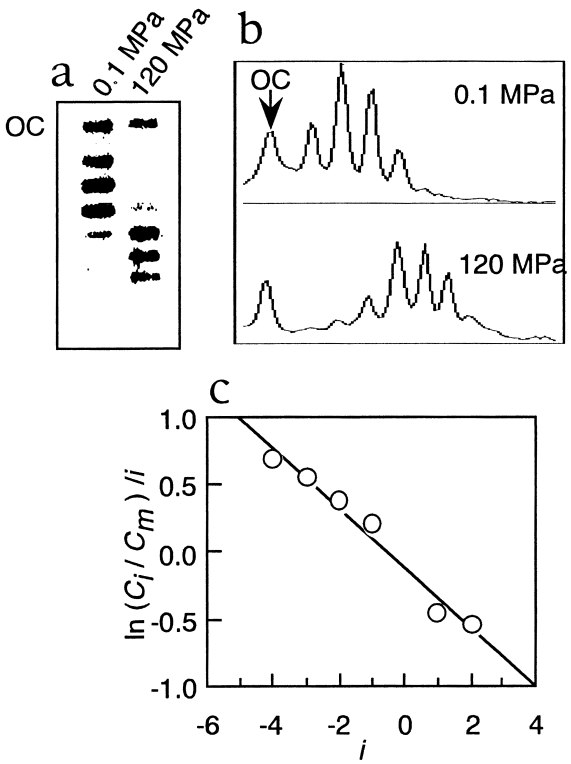
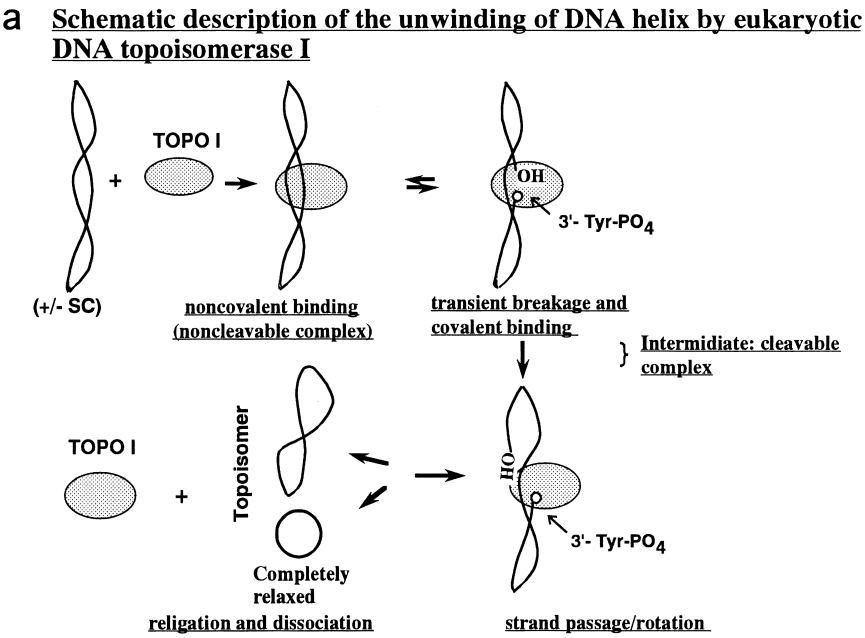
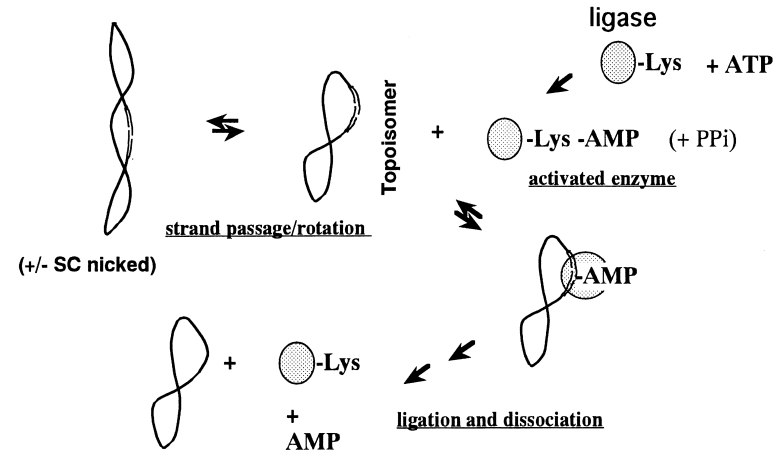


Fig. 1. Typical quantitative analysis of the equilibrium distribution of topoisomers.
(a) Agarose gel electrophoresis shows the distribution of topoisomers generated through T4 DNA ligase I-mediated religation of the singly nicked pBR322 DNA under 0.1 or 120 MPa.
(b) A gel scan of the topoisomer distributions in (a).
(c) A linear regression line plotted to derive parameters of a Gaussian distribution of topoisomers.
OC denotes open-circular form.





Scheme 1.

(constant) in a given medium at a defined temperature and pressure. In practice, we plot $-\ln (C_i/C_m)/i$ as a linear function of i and derive both the distribution center (ω_T) and the torsional energy factor (K). A typical example is shown in Fig. 1.

Results and Discussion

Topoisomer Distributions under Elevated Pressure. Eukaryotic DNA topoisomerase I and T4 DNA ligase, which were used in our supercoiling assay for pressure effects on the DNA topological structure, introduce DNA topoisomers through different catalytic routes (Scheme 1). DNA topoisomerase catalyzes the unwinding of closed-circular DNA duplexes through cycles of transiently nicking one strand of DNA and rejoining the nick without consumption of additional energy, whereas T4 DNA ligase utilizes the energy of ATP to ligate neighboring 5′-P and 3′-OH ends of DNA. Resealing of the nicked strand results in different linking numbers in the closed-circular forms created from the correspondingly different twisting values of the nicked circular DNA molecules.

First, possible effects of increasing pressure on the enzyme structure/activity of T4 DNA ligase were examined by comparing the effects of preincubation under elevated (200 MPa) or under ambient pressure on the enzyme for its relaxation capability after high pressure release (data not shown), as was done previously with wheat germ topoisomerase I.[16] Similar to DNA topoisomerase I, no essential discrepancy was found in the topoisomer distributions of DNA by the pressurized preincubation. Furthermore, we observed that the employment of elevated pressure on supercoiling assays by the two different enzymes had equal consequences. It was therefore expected that the main effects of elevated pressure here were relevant to DNA topological structures, rather than the activity of a specif-

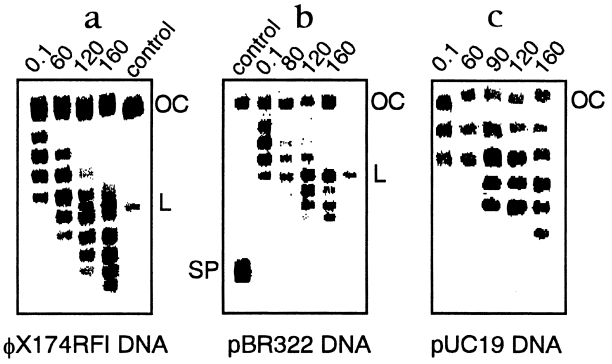


Fig. 2. One-dimensional agarose gel electrophoretic analysis of pressure effects on topoisomer distribution.
(a) Topoisomerase I-mediated relaxation of ϕX174RFI DNA molecules singly nicked by DNase I.
(b) T4 DNA ligase-mediated ligation of linearized pBR322 DNA molecules (by EcoRI restriction endonuclease).
(c) Topoisomerase I-mediated negatively supercoiled pUC19 DNA molecules.
Conditions for relaxation reactions and electrophoretic analysis are described in the methods.
OC, L, and SP denote open-circular, linear, and negatively supercoiled form, respectively.

ic enzyme. We focus our attention on the response of DNA topological structure to pressure increase in the presence of DNA topoisomerase/ligase in the following discussion.

Figures 2 and 3 show the typical results of one and two dimensional agarose gel electrophoretic analysis of the topoisomer distributions resulting from reactions performed with topoisomerase I or ligase under ambient and elevated hydrostatic pressures. Under normal conditions, the equilibrium distribution of topoisomer products may be recovered using a Gaussian model.[6,7] If the exposure of DNA to certain substances (e.g., DNA-binding proteins/ligands) or physical variables (e.g., temperature, ions, or pressure) gives rise to modifications in the geometry of the DNA helix, these modifications can be disclosed by evaluating subsequent alterations in the topoisomerase distribution patterns ex situ. Accordingly, progressive increases in the gel migration mobility of the major topoisomers of ϕX174RFI DNA, as shown in Figs. 2 and 3, indicate that high pressures altered the superhelical conformation. It appeared that the application of pressure also widened the topoisomer distribution in some cases of topoisomerase I-mediated unwinding of supercoiled DNA.

Negative or positive topoisomers with identical absolute values of *Lk* have equal gel mobility in 1-D electrophoresis in the absence of intercalating drugs or certain divalent metal ions. Unambiguous discrimination of these topoisomers can be achieved using two-dimensional electrophoresis, following reaction with intercalating drug molecules. During 2-D electrophoresis, binding with chloroquine causes topoisomers originally with positive supercoiling to obtain further positive superhelicity so that they move faster, while the originally negatively supercoiled forms lose negative superhelicity and move slower.[8] Differentiation in the gel mobility of DNA-drug complexes can separate the original negative and positive DNA onto the two sides of the arch formation. We observed that topoisomer products of ϕX174RFI DNA resulting either from topoisomerase I- or from T4 DNA ligase-mediated relaxation appeared in the right half arch, indicating that the supercoiling assay conducted under these conditions produced positively supercoiled products. Furthermore, greater gel mobility was found for the topoisomers resulting from the pressurized unwinding, indicating the increased superhelicity of topoisomers with positive supercoiling.

Effects of Pressure on Linking Number, Rotation Angle, and Torsional Free Energy Factor. Several experiments, involving various DNA species, similar to those described above (Figs. 1a, 2, and 3) were conducted under ambient and elevated pressures and analyzed by one- or two- dimensional agarose gel electrophoresis. Alterations in the linking numbers of major topoisomers were quantified by analysis of the shifts in topoisomer distribution according to the band-counting method.[19] The corresponding linking number differences, ΔLk, relative to those at 0.1 MPa, in individual gels were plotted against the pressure. As shown in Fig. 4, differences in the linking numbers (ΔLk) as a fraction of turns of the distribution centers were expressed in terms of dependence on hydrostatic pressure. Assuming a linear relationship between ΔLk and hydrostatic pressure, the slope ($S = \Delta Lk/\Delta P$) can be calculated and related to variations in the average helix rotation angles Ω (angular degree); $\Omega = 360^{*}S/N$. The value of Ω reflects the effect of agents on the helix rotation of DNA molecules (a positive value indicates winding of the DNA double helix). The resulting Ω values are displayed in Table 1, together with previously determined data for comparison. Pressure effects on winding of the DNA duplex appear to be independent of DNA species but varied slightly with ionic conditions. Data in Table 1 suggest that a stress of 100 MPa may result in increases in the DNA supercoiling density by 0.002 for pUC19 DNA (2686 bps), 0.004 for pBR322 DNA (4361 bps), or 0.005 for ϕX174RFI DNA (5386 bps) under conditions of 10 mM Mg^{2+} and 50 mM Na^{+} at 20 °C. Overwinding of the DNA double helix at elevated pressures within the physiological range suggests potential biological effects.

It is generally accepted that K is a constant in a given medium and is independent of the DNA species when the DNA is longer than 2.5 kb.[5,8,15,21–27] For DNA molecules with fixed lengths, K appears to vary only with intrinsic properties of the DNA, such as torsional rigidity, persistent length, and/or effective diameter. Figure 5 depicts the effect of elevating the pressure on determinations of K, reflecting the free energy of DNA supercoiling averaged to changes per linking unit. Experimental errors in the measurement of K are assumed to be less than 10%. Increasing the pressure appeared to lower the value of K in the presence of topoisomerase I or T4 DNA ligase, in DNA relaxation reactions.

Determining the slope of the resulting regression line involves reconciliation between the effects of various environmental conditions (e.g., pressure, temperature, and ionic strength and type of ion) on the torsional energies of the DNA. Assuming a linear relationship between experimental values of K and the pressure, we can estimate values of $(\partial K/\partial P)_T$ to be 2.1, 1.4, and 1.8 MPa^{-1}, corresponding to metal ion concentrations of 50 mM Na^{+}, 10 mM Mg^{2+}, and 50 mM Na^{+} and 10 mM Mg^{2+}, respectively. Several experiments and theoretical

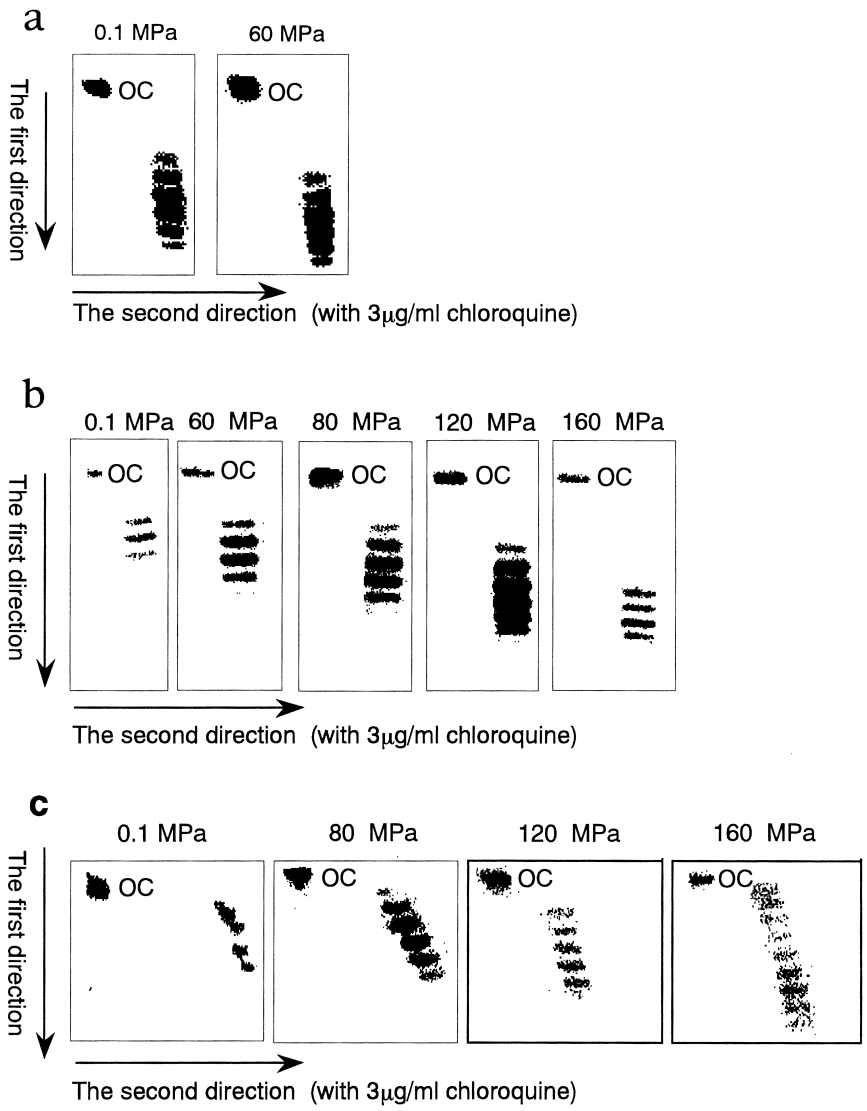


Fig. 3. Two-dimensional gel electrophoretic analysis of pressure effects on topoisomer distribution.
(a) Topoisomerase I-mediated relaxation of pBR322 DNA.
(b) T4 DNA ligase-mediated ligation of linearized ϕX174RFI DNA.
(c) Topoisomerase I-mediated relaxation of negatively supercoiled ϕX174RFI DNA.
Conditions for relaxation reactions and electrophoretic analysis are described in the methods.
OC denotes open-circular form.

simulations have found decreases in the values of K with increases in the temperature[10,26,27] or the ionic strength.[13] Given the limited range of ionic conditions evaluated in the present study, further experiments are required to elucidate a relationship between the ionic and hydrostatic conditions with regard to their effects on topological conformations of DNA. Separate analysis of the function of K with the same pressure at different salt concentrations gives comparable values, such as 1237 in the case of 50 mM Na^+ and 1184 in the case of 10 mM Mg^{2+} and 50 mM Na^+. Alternatively, the observation that the values of K at identical pressures but under different ionic conditions fluctuate within the same range of experimental uncertainty suggests that the average slope of -1.8 MPa^{-1} may appropriately reflect the relationship between K and P (the line in Fig. 5). By locating the ordinate of the assumed regression line in Fig. 5, the averaged value of K at 0.1 MPa was estimated to be 1207 (an arrow in Fig. 5).

The free energy of DNA supercoiling can be described by the following equation:[5,6,15,28]

$$\Delta G = KRT(\Delta Lk)^2/N, \quad (2)$$

where R is the gas contant. In principle, one may expect that volume changes upon DNA supercoiling could be derived from the relationship between the free energy and the pressure, $[\partial(\Delta G)/\partial P]_T = \Delta V$, at a constant temperature for DNA molecules with a given superhelical state. We instead discuss changes in the values of K in response to pressure in the form of $(\partial K/\partial P)_T$ since K reflects the free energy for changing one linking number unit (i.e., $Lk_n \rightleftarrows Lk_{n-1}$) of a given topoisomer. In our opinion, this may be an appropriate way to associate the supercoiling density with the space density of closed-circular

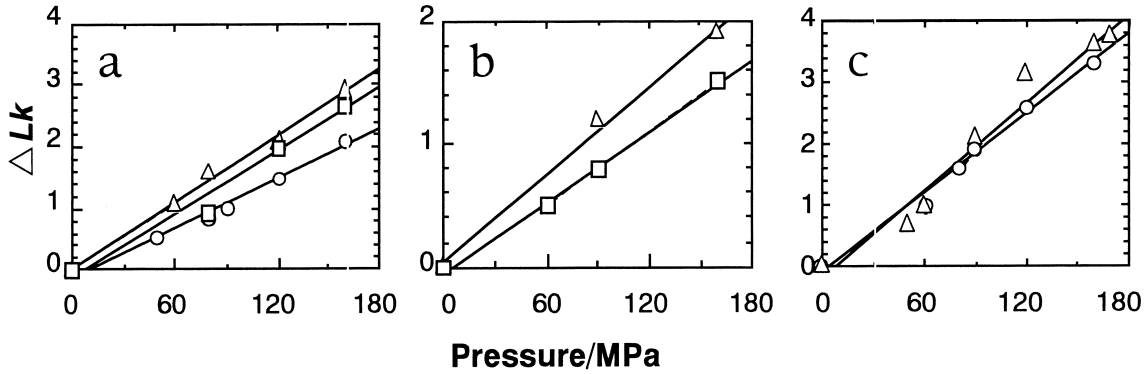


Fig. 4. Pressure dependence of linking number differences (ΔLk) of ϕX174RFI (a), pBR322 (b), and pUC19 DNA (c), respectively.
Linking numbers (Lk) were obtained at different metal salt concentrations.
(○) 50 mM NaCl, through topoisomerase I-mediated relaxation.
(□) 10 mM $MgCl_2$ through topoisomerase I-mediated relaxation.
(△) 50 mM NaCl + 10 mM, $MgCl_2$, through T4 DNA ligase-mediated ligation.
Other conditions; 50 mM Tris-HCl, (pH 7.5) and 20 °C. 5% Glycerol was added for topoisomerase. 1 mM ATP and 1mM DTT were added for DNA ligase.
Pressure-induced changes in Lk were counted as compared with the reference values at 0.1 MPa. Salt effects on Lk at 0.1 MPa were not normalized.

Table 1. Variations in the Average Helix Rotation Angles Ω (in 10^{-3} Angular Degree/Base/MPa) of DNA Molecules Caused by the Application of Hydrostatic Pressure at 20 °C

DNA molecules	Salt concentrations		
	50 mM NaCl	10 mM $MgCl_2$	10mM $MgCl_2$ + 50mM NaCl
pBR322	1.1[a)]	1.4	1.7[a)]
PUC19	N.D.[b)]	1.3	1.6
ϕX174RF I	N.D.[b)]	1.4	1.6

a) Data extracted from Ref. 16.
b) N.D., not determined.

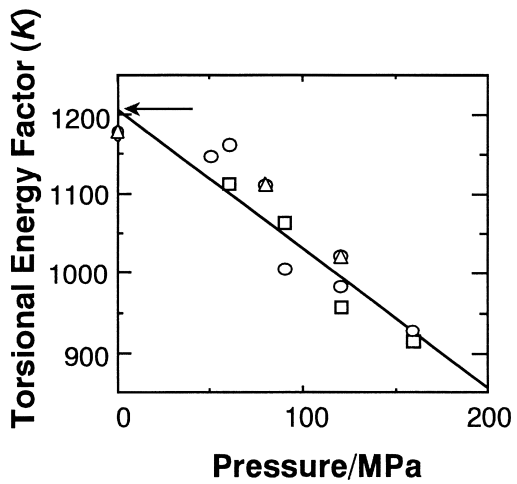


Fig. 5. Pressure dependence of the torsional energy factor (K).
Symbols are as in Fig. 4.

DNA in an evaluation of the pressure dependence of DNA supercoiling. Furthermore, a tentative expression, Δv may be introduced to discuss variances in reaction volumes related to winding of the DNA double helix as expressed by:

$$\Delta v = (RT/N)(\partial K/\partial P)_T. \quad (3)$$

Despite the current state of ignorance of how these changes occur as pressure increases, putative estimates of volume variances by increasing unit of linking numbers at 20 °C can be obtained by inserting the averaged value of $(\partial K/\partial P)_T$ into Eq. 3. Supposing the identically minimal compressibility of all topoisomers, we found that Δv is approximately $-4.4/N$ M^{-1} (in base pairs). Negative values of volume changes upon increasing by one unit of DNA supercoiling appear to be in agreement with the notion that highly supercoiled DNA is in a more compact and interwound state.[29–31] Generally, changes in volume may be attributed to structural changes in the supercoiled DNA as well as to variations in hydration and counter ion equilibria accompanied with changes in the winding states of the DNA helix. Given the current lack of quantitative descriptions of real volumes of supercoiled DNA at various winding states and the high sensitivity of the structure of supercoiled DNA to the environment, it is difficult to ascribe a particular volume change to a specific process.

The effects of pressure on DNA supercoiling suggest a relationship with the thermal equilibrium of topoisomer linking number distributions. At elevations in pressure up to 200 MPa, the compressibility of DNA molecules is minimal and the sta-

bility of the stacked structure of the base pairs is generally improved.[32] For synthetic DNA polymers with a simple repeating unit of d(*A*·*T*), Fourier transform IR spectroscopic signals were consistent with pressure-induced enhancement in DNA hydration and base pairing.[33] It appeared that, under moderately elevated pressure (e.g., $<$ 200 MPa), an increase in the DNA hydration was involved in the Z-B structural transition of poly[d(G–C)],[33] whereas a further increasing pressure (e.g., up to 600 MPa), combined with high salt concentration (5 M NaCl), probably aroused the structural changes in water itself, which was suggested to be responsible for the B- or A- to -Z structural transitions of oligonucleotides.[17,18] There may be certain connections between the stabilization of base stacking, by increasing the pressure, increasing the ionic strength or lowering the temperature, and the effect of DNA helix winding parameters, though the mechanisms of the effects of ionic strength and temperature on DNA supercoiling are not identical. To determine the origins of thermal fluctuations in *K* of supercoiling, Schurr and colleagues proposed a model involving an equilibrium of different secondary structural intersubunits with different twisting energy values and torsional rigidity values.[34,35] It is possible that the distributions of DNA structures and energy basins are open to disturbances in other environmental factors, and may ultimately be attributed to some equilibrium based on the reconciliation of changes in these various environmental parameters. Interactions between DNA segments that are reflected by the effective helix diameter are significantly affected by ionic conditions since binding of counter ions with DNA effectively shields repulsive forces between contacting segments. High pressure discourages ion bonding while favoring the exposure of more charged groups to the solvent in aqueous solutions in order to solvate by more water (electrostriction effect). The variable pressure dependence of the DNA torsional energy factor under different ionic conditions implies that pressure effects on DNA supercoiling may be partly related to electrostatic interactions between DNA segments and counter ions. There are numerous challenging problems concerning the exact behavior of DNA structural parameters, which may be dependent upon ionic and/or thermal conditions, under the stress of high hydrostatic pressure. The structure and dynamics of nucleic acids, as a special class of biopolymers, under pressure conditions are far from fully explored.

The finding that moderately elevated pressure induced overwinding of the DNA duplex has biological implications for the environmental adaptations of submarine microorganisms subjected to high pressure and high or low temperatures.[36–38] The stress of elevated hydrostatic pressure has been found to stabilize the cells and enzymes of some thermophilic organisms against the negative effects of high temperature.[36,38–40] Furthermore, taking into account the flexibility of the tertiary structure of supercoiled DNA may be useful for the emerging study on gene and cell activity under artificial conditions such as elevated hydrostatic pressure.[41–43]

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References

1 J. C. Wang, *Ann. Rev. Biochem.*, **65**, 635 (1996).
2 K. Drlica, *Mol. Microbiol.*, **6**, 425 (1992).
3 Y.-C. Tse-Dinh, H. Qi, and R. Menzel, *Trends Microbiol.*, **5**, 323 (1997).
4 L.-G. Purificacion, S. Knapp, R. Ladenstein, and P. Forterre, *Nucleic Acids Res.*, **26**, 2322 (1998).
5 R. E. Depew and J. C. Wang, *Proc. Natl. Acad. Sci. USA*, **72**, 4275 (1975).
6 D. Pulleyblank, M. Shure, D. Tang, J. Vinograd, and H.-P. Vosberg, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4280 (1975).
7 P. Anderson and W. Bauer, *Biochemistry*, **17**, 594 (1978).
8 C.-H. Lee, H. Mizusawa, and T. Kakefuda, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2838 (1981).
9 C. F. Higgins, C. J. Dorman, D. A. Stirling, L. Waddeu, I. R. Booth, G. May, and E. Bremer, *Cell*, **52**, 569 (1988).
10 M. Duguet, *Nucleic Acids Res.*, **21**, 463 (1993).
11 F. J. Mojica, F. Charbonnier, G. Juez, F. Rodriguez-Valera, and P. Forterre, *J. Bacteriol.*, **176**, 4966 (1994).
12 O. Krispin and R. L. Allmansberger, *FEMS. Microbiol. Lett.*, **134**, 129 (1995).
13 V. V. Rybenkov, A. V. Vologodskii, and N. R. Cozzarelli, *Nucleic Acids Res.*, **25**, 1412 (1997).
14 Y.-C. Xu and H. Bremer, *Nucleic Acids. Res.*, **25**, 4067 (1997).
15 A. V. Vologodskii and N. R. Cozzarelli, *Annu. Rev. Biophys. Biomol. Struct.*, **23**, 609 (1994).
16 G.-Q. Tang, N. Tanaka, and S. Kunugi, *Biochim. Biophys. Acta*, **1443**, 364 (1998).
17 A. Krzyzaniak, P. Salanki, J. Jurcazk, and J. Barciszewsk, *FEBS. Lett.*, **279**, 1 (1991).
18 A. Krzyzaniak, J. P. Furste, V. A. Erdmann, P. Salanski, J. Jurczak, and J. Barciszewski, *Biochimie*, **78**, 862 (1996).
19 W. Keller, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4876 (1975).
20 G.-Q. Tang, N. Tanaka, and S. Kunugi, *Chem. Lett.*, **1997**, 717.
21 T. Schlick, B. Li, and W. K. Olson, *Biophys. J.*, **67**, 2146 (1994).
22 A. Seidl and H. J. Hinz, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 1312 (1984).
23 D. Shore and R. L. Baldwin, *J. Mol. Biol.*, **170**, 983 (1983).
24 D. S. Horowitz and J. C. Wang, *J. Mol Biol.*, **173**, 75 (1984).
25 W. R. Bauer and C. J. Benham, *J. Mol. Biol.*, **234**, 1184 (1993).
26 A. N. Naimushin, J. B. Clendenning, U. S. Kim, L. Song, B. S. Fujimoto, D. W. Stewart, and J. M. Schurr, *Biophys. Chem.*, **134**, 129 (1995).
27 W. R. Bauer, H. Ohtsubo, E. Ohtsubo, and C. J. Benham, *J. Mol. Biol.*, **253**, 438 (1995).
28 W. R. Bauer and J. Vinograd, *J. Mol. Biol.*, **47**, 419 (1970).
29 J. Bednar, P. Furrer, A. Stasiak, J. Dubochet, E. H. Egelman, and A. D. Bates, *J. Mol. Biol.*, **235**, 825 (1994).
30 J. A. Gebe, J. J. Delrow, P. J. Heath, B. S. Fujimoto, W. Stewart, and J. M. Schurr, *J. Mol. Biol.*, **262**, 105 (1996).
31 Y. L. Lyubchenko, and L. S. Shkyakhtenko, *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 496 (1997).
32 S. A. Hawley and R. M. Macleod, *Biopolymers*, **13**, 1417 (1974).
33 R. B. Macgregor Jr, *Biopolymers*, **48**, 253 (1998).
34 J. F. Derlow, P. J. Heath, and J. M. Schurr, *Biophys. J.*, **73**,

2688 (1997).
35 J. M. Schurr, J. F. Derlow, B. S. Fujimoto, and A. S. Benight, *Biopolymers*, **44**, 283 (1998).
36 G. N. Somero, *Annu. Rev. Physiol.*, **54**, 557 (1992).
37 D. Bartlett, C. Kato, and K. Horikoshi, *Res. Microbiol.*, **146**, 697 (1995).
38 J. W. Deming, *Curr. Opin. Biotechnol.*, **9**, 283 (1998).
39 M. Summit, B. Scott, K. Nielson, E. Mathur, and J. Baross, *Extremophiles*, **2**, 339 (1998).
40 F. Canganella, J. M. Gonzalez, M. Yanagibayashi, C. Kato, and K. Horikoshi, *Arch. Microbiol.*, **168**, 1 (1997).
41 D. H. Barttelt, *Nature*, **342**, 572 (1989).
42 J. Klein-Nulend, J. Roelofsen, C. M. Semeins, A. L. Bronckers, and E. H. Burger, *J. Cell. Physiol.*, **170**, 174 (1997).
43 K. Karniranta, M. Elo, R. Sironen, M. J. Lammi, M. B. Goldring, J. E. Eriksson, L. Sistonen, and H. J. Helminen, *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 2319 (1998).