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Evidence that a Plasmid from a Hyperthermophilic Archaebacterium Is Relaxed at Physiological Temperatures

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A plasmid of 3.45 kb (pGT5) was recently discovered in a strain of hyperthermophilic archaebacterium which was isolated from samples collected in a deep-sea hydrothermal vent. This strain (GE5) grows within a temperature range of 68 to 101.5°C, and we show here that it contains a strong ATP-dependent reverse gyrase activity (positive DNA supercoiling). By comparison with eubacterial plasmids of known superhelical densities, we estimated the superhelical density of the archaebacterial plasmid pGT5 to be −0.026 at 25°C. The equation which relates the change of the rotation angle of the DNA double helix with temperature was validated at 95°C, the optimal growth temperature of the GE5 strain. Considering these new data, the superhelical density of plasmid pGT5 was calculated to be −0.006 at the physiological temperature of 95°C, which is close to the relaxed state. This finding shows that the DNA topology of a plasmid isolated from a hyperthermophilic archaebacterium containing reverse gyrase activity is strikingly different from that of typical eubacterial plasmids.

In eukaryotes and mesophilic eubacteria, the DNA topoisomerases either relax or introduce negative superturns into DNA (10, 39). On the contrary, in extreme thermophilic archaebacteria, the major DNA topoisomerase activity is due to reverse gyrase. This is an ATP-dependent type I DNA topoisomerase which introduces positive superturns (5, 7, 16, 21, 27, 29, 36). This finding raised the possibility that DNA is positively supercoiled in order to be protected against thermal denaturation; hence, there is special interest in the study of DNA topology in these microorganisms.

It has been shown previously that the DNA of the extreme thermophilic archaeobacteriophage SSV1 is positively supercoiled (28), unlike all other natural DNA. For a long time, the SSV1 genome was the only available DNA isolated from an extreme thermophilic archaebacterium suitable for topological analysis. Our recent discovery of a plasmid of 3.45 kb, called pGT5, in a new hyperthermophilic sulfur-metabolizing archaebacterium (GE5 strain) (12) has provided another DNA suitable for such studies. The GE5 strain was isolated from samples collected in a deep-sea hydrothermal vent in the North Fiji Basin (1). This strain is strictly anaerobic and grows within the temperature range of 68 to 101.5°C. It has a generation time of 34 min at the optimal growth temperature of 94°C. The GE5 strain fulfills the criteria already proposed for an archaebacterium; the cells possess both di- and tetraether lipids and are resistant to chloramphenicol, penicillin, vancomycin, and streptomycin (12).

We report here the topological analysis of the plasmid pGT5. Our results suggest that pGT5 is relaxed at physiological temperatures, although a strong reverse gyrase activity is detected in the GE5 strain.

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MATERIALS AND METHODS

Chemicals and enzymes. Ethidium bromide, chloroquine, and phenylmethylsulfonyl fluoride were purchased from Sigma Chemical Co.; *Eco*RI, bovine pancreatic DNase I, leupeptin, pepstatin, and serum albumin were from Boehringer (Mannheim, Germany); ethylene glycol and dithiothreitol were from Prolabo; and Indubiose A37NA was from IBF (Villeneuve-la-Garenne, France). All the other chemicals were from Merck (Darmstadt, Germany).

Plasmid purification. The conditions for growing GE5 cells will be described in detail elsewhere (13). Briefly, anaerobic cultures were cultivated at different temperatures in YPS medium in a gyrotary shaking incubator. YPS medium is made of artificial seawater supplemented with trace elements, 0.1% yeast extract, 0.4% peptone, and 0.5% elemental sulfur (S^0) at pH 6.8. Cells were lysed at room temperature by the addition of 1% *N*-lauroyl sarcosine and 1% sodium dodecyl sulfate. Proteolysis was caused by treatment with 1 mg of proteinase K per ml at 50°C for 3 h and stopped by the addition of 1 mM phenylmethylsulfonyl fluoride at room temperature. After centrifugation at 10,000 rpm for 15 min in a Beckman JS13 rotor, the DNA was precipited by 100% ethanol. The pellet was dried, suspended in 1 ml of buffer B (10 mM Tris-HCl [pH 8.0], 2 mM EDTA), and treated with 20 μg of RNase per ml at 37°C for 1 h. Isolation of the plasmidic DNA was achieved by a cesium chloride gradient as described elsewhere (24).

Plasmidic and substrate DNAs. pBR322 (from *Escherichia coli* HB101) was purchased from Pharmacia LKB. pTZ18 was isolated from *E. coli* JM109 growing at 37°C, as described by Maniatis et al. (24). Covalently closed circular pTZ18 (form I) was converted to the open circular form (form II) by limited pancreatic DNase I digestion in the presence of ethidium bromide (2). The ligation procedure using DNA ligase from *Thermus thermophilus* was performed according to the method of Jaxel et al. (19). The linear form (form III) was prepared by *Eco*RI digestion of

pTZ18. The plasmid pTZ18 (form I) was partially relaxed by incubating 500 ng of DNA with 0.7 enzymatic unit of eukaryotic DNA topoisomerase I for 5 min at 37°C in a reaction mixture containing 50 mM Tris-HCl (pH 7.8), 80 mM NaCl, 10 mM $MgCl_2$, 50 μg of bovine serum albumine per ml, 0.5 mM dithiothreitol, and 0.1 mM EDTA. One enzymatic unit of DNA topoisomerase relaxes 100% of negatively supercoiled DNA in 5 min at 37°C.

Two-dimensional electrophoresis. Electrophoresis in the first dimension was performed in 0.7% agarose gels for 16 h at 2 V cm^{-1} in 90 mM Tris-borate–2 mM EDTA, pH 8.0 (TEB buffer). After electrophoresis, the gel was soaked for 8 h at room temperature in TEB buffer containing chloroquine. Electrophoresis in the second dimension was performed in the same buffer containing chloroquine for 16 h at 1.3 V cm^{-1}. The gel was stained with ethidium bromide, and chloroquine was eliminated with $MgSO_4$ (1 mM). Polaroid photographs were taken under 254-nm UV illumination.

Densitometric analysis. Made with a Sony (Paris, France) CCD video camera, polaroid photographs were digitized by the use of Azur software on a Macintosh Apple computer having a Photon acquisition card. The software and card were purchased from Orkis (Aix-en-Provence, France). The image was analyzed by the use of Scan Analysis software by Biosoft (Cambridge, England) in the manual mode, in which each visible DNA topoisomer was considered.

Detection of reverse gyrase activity. Cell crude extracts from the GE5 strain were prepared, with some modifications, as described elsewhere (5). Cells were suspended in a Tris-HCl (pH 8.0) buffer containing 1.5 M NaCl; 1 mM (each) dithiothreitol EDTA, EGTA, and phenylmethylsulfonide fluoride; and 5 μg each of leupeptin and pepstatin per ml. Ammonium sulfate and sodium bisulfite were omitted at this step, unlike the previously published method (5). Cells were lysed by addition of 0.5% Triton X-100 for 1 h at 4°C. After centrifugation at 13,000 rpm for 30 min in a Beckman JS13 rotor, the supernatant (GE5 crude extract) was carefully removed. Reverse gyrase activity was assayed by incubating 500 ng of pTZ18 DNA with 25 ng of proteins from GE5 crude extract for 15 min at 94°C. The standard reaction mixture contained 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM $MgCl_2$, 30 μg of bovine serum albumine per ml, 12% ethylene glycol, 0.5 mM dithiothreitol, and 0.5 mM EDTA.

RESULTS

Estimation of the pGT5 DNA topology by comparison with eubacterial plasmids pBR322 and pTZ18. In a preliminary experiment, we observed that the plasmid pGT5, isolated from cells cultivated at 95°C, was relaxed during agarose gel electrophoresis in the presence of chloroquine (2.5 μg/ml) or ethidium bromide (10 ng/ml). Since these two DNA intercalating drugs relax only negatively supercoiled DNA (30, 38), this indicated that plasmid pGT5 was negatively supercoiled at the temperature of the experiment (25°C).

To further elucidate the pGT5 DNA topology, we analyzed this plasmid, along with the two eubacterial plasmids (pTZ18 and pBR322) as controls, by two-dimensional electrophoresis. In order to visualize the set of topoisomers present in the forms I of these plasmids in the first electrophoresis, chloroquine was added at a concentration known to relax eubacterial plasmids (2.5 μg/ml). A higher concentration of chloroquine (5 μg/ml) was added in the second electrophoresis to separate the topoisomers which were either positively or negatively supercoiled in the first migration. Figure 1A shows that the two eubacterial plasmids pTZ18 and pBR322 (lanes B and D, respectively) have been relaxed by chloroquine but are still negatively supercoiled at 25°C (temperature of the electrophoresis). We determined by densitometry (Fig. 1B) that the major topoisomer of pTZ18 and pBR322 contains 6 ± 0.5 and 9 ± 0.5 negative superturns, respectively. On the other hand, the archaebacterial plasmid pGT5 has been positively supercoiled by the addition of chloroquine in the gel, with a major topoisomer containing 2 ± 0.5 positive superturns.

In an independent experiment, the superhelical density of the plasmid pTZ18 was calculated, according to the band counting method described by Keller (20), to be −0.052 at 25°C. This value was used to calculate superhelical densities of pBR322 and pGT5 at 25°C from the data of Fig. 1; the results were −0.051 and −0.026, respectively.

Effect of high temperatures on DNA supercoiling. In order to determine the topological state of the archaebacterial plasmid pGT5 in the physiological conditions, the temperature effect on DNA supercoiling needs to be considered. According to the equation $Lk = T + W$ (8), negative superturns ($W < 0$) were expected to have been introduced in pGT5 when the temperature decreased from 95°C (culture medium) to 25°C (electrophoresis), thus keeping the linking number (Lk) constant when the number of helix turns (T) increases. In order to evaluate the effect of very high temperatures on DNA supercoiling, we closed open circular pTZ18 plasmids using the thermophilic DNA ligase from *T. thermophilus* (37) at temperatures from 25 up to 95°C (Fig. 2A) and determined by densitometry the value and sign of each major topoisomer (Fig. 2B). The data of Fig. 2C show that the variation of the linking number with temperature is constant from 25 to 95°C. From these data, we estimated that the change of the rotation angle of the DNA double helix with temperature is −0.010°/°C/bp, within the range of tested temperatures. This is in agreement with the values previously determined at lower temperatures (13 to 41°C) by two laboratories: −0.010° and −0.014° ± 0.001°/°C/bp (references 9 and 31, respectively). A similar result has been obtained with different plasmids at temperatures up to 90°C (11).

pGT5 superhelical density at physiological temperatures. Taking into consideration the above-mentioned result, we calculated that the actual superhelicity at 95°C of the plasmid pGT5 is −0.006. In order to investigate the effect of growth temperature on pGT5 topology, we tried to purify the pGT5 DNA from cells cultivated at different temperatures. We determined the superhelical densities of pGT5 isolated from cells grown at 80 and 85°C to be −0.0125 and −0.011, respectively (6). The values for the pGT5 superhelical density calculated at 80, 85, and 95°C are very different from the values of −0.049 and −0.048 for the superhelical densities of pTZ18 and pBR322 at the *E. coli* physiological temperature (37°C). The latter values are in agreement with those reported in the literature for eubacterial plasmids (3). Our results therefore indicate that the isolated pGT5 is very close to the relaxed state at physiological temperatures.

Because cell growth is very low at temperatures below 80°C and above 95°C, we cannot obtain at such temperatures enough plasmid DNA suitable for topological analysis. Nevertheless, these preliminary results seem to indicate that growth temperature has an effect on DNA topology, in that an increase of the GE5 growth temperature induces a decrease in the pGT5 negative supercoiling.

Reverse gyrase activity in crude extracts from strain GE5. Since positive supercoiling in the extreme thermophilic archaebacteria is supposed to be related to the action of a

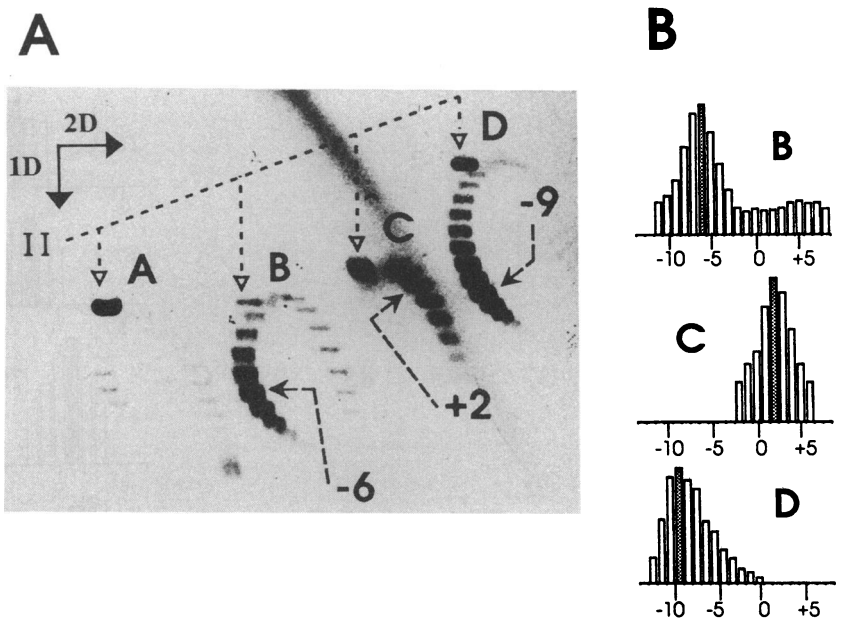

FIG. 1. Comparative topoprofiles of pTZ18, pGT5, and pBR322 analyzed by two-dimensional agarose gel electrophoresis in the presence of chloroquine in both dimensions (A) and densitometric analysis (B). (A) Arrows 1D and 2D show the direction of the run in the first and second electrophoreses at 25°C. Lanes B, C, and D correspond to the plasmids pTZ18, pGT5, and pBR322, respectively. The plasmids were relaxed during the first electrophoresis with 2.5 μg of chloroquine ml^{-1}, and topoisomers with opposite signs were separated during electrophoresis in the second dimension by the addition of 5 μg of chloroquine ml^{-1}. Lane A corresponds to the plasmid pTZ18 partially relaxed by chicken DNA topoisomerase I. Without chloroquine, this DNA is partially relaxed with a majority of negatively supercoiled topoisomers. When chloroquine is present during the first electrophoresis, this DNA is composed of a majority of positively supercoiled topoisomers. This control indicates that the left branches of arches A, B, and D correspond to negatively supercoiled topoisomers, whereas the right branches of arches A and C correspond to positively supercoiled topoisomers. Arrows indicate the value and the topological sign (−6, +2, and −9) of the major topoisomer of each plasmid and the open circular form II DNA (II). (B) Densitometric analysis effected as described in Materials and Methods. DNA topoisomers values and signs are presented in the abscissa scale.

reverse gyrase, an obvious explanation for the relaxed topological state of the plasmid pGT5 could have been the absence of reverse gyrase in the GE5 strain.

We have therefore investigated the existence of a reverse gyrase activity in the GE5 strain. Figure 3 shows the products of incubation of negatively supercoiled pTZ18 DNA with GE5 crude extract. We analyzed by two-dimensional electrophoresis the conversion of pTZ18 DNA substrate (lane A) to a set of topoisomers (lane D). In these gels, the left branch of the arch represents negatively supercoiled topoisomers while the right branch is made up of positively supercoiled DNA. As a control, we prepared relaxed pTZ18 DNA at 94°C, incubating open circular pTZ18 molecules with thermophilic DNA ligase; the migration of this product is shown in lane B.

In a reaction which is strictly ATP dependent (compare lanes C and D), the negatively supercoiled pTZ18 DNA became completely positively supercoiled after incubation at 94°C with cell crude extracts from the GE5 strain. Positive supercoiling was observed from 10 μM to 1 mM ATP, which is characteristic of reverse gyrase activity (16).

In the standard reaction with 1 mM ATP and 1.5 ng of protein per μl from GE5 crude extracts, pTZ18 DNA became positively supercoiled with a major topoisomer containing at least 6 ± 0.5 positive superturns. This corresponds to a superhelical density of +0.035 at 94°C. Positive supercoiling was still observed with cell crude extracts containing only 0.15 ng of protein per μl, suggesting a very strong reverse gyrase activity in strain GE5.

A low DNA-relaxing activity can be observed in the absence of ATP (lane C); this very low activity is typical for extreme thermophilic archaebacteria. On the other hand, this ATP-independent relaxation is the only activity detected in mesophilic and moderately thermophilic archaebacteria (5) and the major activity detected in extreme thermophilic eubacteria (4).

DISCUSSION

Plasmid pGT5 is the first plasmid isolated from a hyperthermophilic sulfur-metabolizing archaebacterium. Two extrachromosomal DNAs were previously detected in sulfolobales which are extremely thermophilic aerobic archaebacteria but not hyperthermophiles: pSSV1 (15 kb) from *Sulfolobus shibatae* (18), previously called *Sulfolobus acidocaldarius* B12 (40), and pSL10 (7 kb) from *Desulfurolobus ambivalens* (42). These extrachromosomal DNAs correspond to lysogenic forms of lemon-shaped archaeobacteriophages SSV1 (25) and DAV1 (41), respectively. No viruslike particles were detected by electron microscopy in cultures of the GE5 strain, suggesting that pGT5 is a real plasmid (13).

It has been previously reported that the DNA isolated from SSV1 is positively supercoiled (28). However, since the SSV1 DNA was recovered after UV irradiation of the cells, it was not clear whether this unusual topological state could be considered representative of the DNA topology in extremely thermophilic archaebacteria. It was thus interesting to analyze the topology of a plasmid such as pGT5.

Our present results suggest that pGT5, isolated from exponentially growing cells, is relaxed at physiological tem-

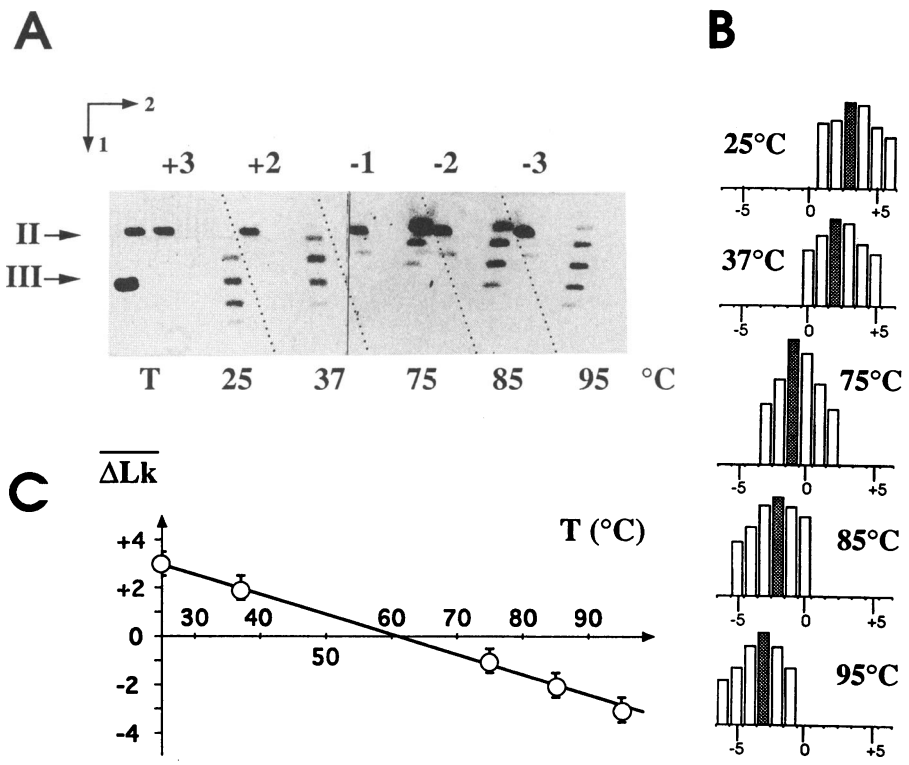

FIG. 2. Effect of high temperatures on the DNA supercoiling. (A) Topoprofiles of pTZ18 covalently closed by the DNA ligase from *T. thermophilus* HB8 at several temperatures. By using this thermophilic enzyme even at low temperatures, it was possible to have the same salt conditions in all ligations. The ligation products were analyzed by two-dimensional electrophoresis. The first electrophoresis was performed without chloroquine at 25°C. Chloroquine (2.5 μg/ml) was added in the second electrophoresis. Lane T represents the migration of the open circular (II) and linear (III) plasmidic forms of pTZ18, which were used as controls. The other lanes correspond to ligation products at the temperatures indicated at the bottom of the gel. The left branches of the arches obtained at 85 and 95°C correspond to negatively supercoiled topoisomers. The right branches of the arches obtained at 25 and 37°C correspond to positively supercoiled topoisomers. The ligation product at 75°C shows the two populations of topoisomers. After electrophoresis at 25°C, the product of the ligation at the same temperature exhibits an increase of three positive superturns instead of being relaxed. This effect, seen in all cases, is due to the difference in ionic strength between the ligation mixture and the electrophoresis buffer and is constant for all tested temperatures of ligation. Each lane is separated from the others by dotted lines, and the sign and value of each major topoisomer, determined by densitometry (B), are indicated at the top. (C) The average linking number variation with temperature. The linking difference of each major topoisomer ($Lk - Lk^0$) was plotted in a function of the temperature. The relative error bar of ±0.5 corresponds to the limit of the accuracy of the band counting method (32). From this curve, we calculated that the change of the rotation angle of the DNA double helix with temperature is −0.010°/°C/bp from 25 to 95°C, considering the slope of the straight line as well as the 2,880-bp size of pTZ18.

pertures. One could consider that the DNA superhelical density changes with physiological conditions and can be modulated in vivo by salt concentration and DNA-binding proteins. However, one has to keep in mind that despite different values reported for their superhelical densities, eubacterial plasmids always exhibit a high level of negative supercoiling, strikingly different from the relaxed state of the archaebacterial plasmid pGT5 (Fig. 1). Interestingly, we have previously found that plasmids from halophilic archaebacteria—which are mesophiles—are also negatively supercoiled (15, 34, 35). More recent data go as far as to suggest that these halophilic plasmids are even more negatively supercoiled than eubacterial ones (6).

In the hyperthermophilic GE5 strain, our results, albeit preliminary, indicate that growth temperature has an effect on the DNA topology of the pGT5 plasmid, where an increase of the GE5 growth temperature results in a decrease in the pGT5 negative supercoiling. Our recent studies of *Haloferax volcanii* also showed a decrease in the negative supercoiling of the pHV11 plasmid when the *Haloferax* growth temperature increases (26). Therefore, in both a hyperthermophilic archaebacterium (GE5) and a halophilic one (*H. volcanii*), a change in growth temperature has the same effect on plasmid topology. On the contrary, it was previously published that in *E. coli* an increase of growth temperature induces an increase in negative supercoiling of the pBR322 plasmid (17). Even though further information on the effect of other environmental changes on plasmid topology is necessary, such differences in the effect of temperature variation could suggest that observations obtained for a eubacterium like *E. coli* cannot be extrapolated to all microorganisms.

We showed here that GE5 strain contains a strong reverse gyrase activity. The superhelical density of pGT5 isolated from the GE5 strain is therefore much lower than would be expected had reverse gyrase been the only activity responsible for the topological state of the DNA in hyperthermophilic archaebacteria. Thus, another mechanism which relaxes pGT5 is expected to exist in vivo. In eubacteria, positive superturns are relaxed by the activity of DNA gyrase, a type II DNA topoisomerase, whereas in eukaryotes they can be relaxed by either DNA topoisomerase I or

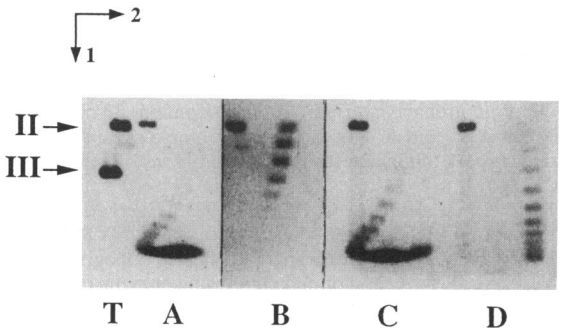

FIG. 3. Detection of the reverse gyrase activity in cell crude extracts from strain GE5. The different incubation products were analyzed by two-dimensional electrophoresis. Procedures were identical to those for Fig. 2A, except that first migration without chloroquine was performed at 2.5 V cm^{-1} and 35°C. Lane A, pTZ18 DNA incubated for 15 min at 94°C without crude extract; lane B, control pTZ18 DNA relaxed at 94°C which had been prepared by ligation of the form II DNA with the thermophilic DNA ligase at the same temperature; lanes C and D, pTZ18 DNA incubated for 15 min at 94°C in the absence (C) or presence (D) of 1 mM ATP. Arrows 1 and 2 show the first and second electrophoresis, respectively. The left branches of the arches in lanes A, B, and C correspond to negatively supercoiled topoisomers, whereas the right branch of the arch in lane D corresponds to positively supercoiled topoisomers. II and III indicate the open circular and linear forms of pTZ18 DNA.

II. The existence of a type II DNA topoisomerase which relaxes both positively and negatively supercoiled DNA has been reported in *Sulfolobus acidocaldarius* (22), but this enzyme is less active and less abundant than reverse gyrase and cannot be detected in crude extracts. The detection of a DNA topoisomerase II activity in crude extracts from GE5 has not been possible either (6). Therefore, it seems unlikely that DNA topoisomerase II is involved in the processes responsible for the relaxed state of pGT5.

An alternative possibility would be that the relaxation of pGT5 is a result of a balancing out of the reverse gyrase activity and the accumulation of negative superturns produced by DNA tracking processes. Normally, these processes, such as DNA transcription, produce both positive and negative superturns which are later relaxed by DNA topoisomerases (23). Negative superturns could accumulate if they are specifically sequestered by the formation of nucleosomelike structures. Upon removal of these structures, during the isolation of plasmids, the negative superturns would be released and could counterbalance the positive superturns introduced in vivo by the reverse gyrase.

The findings reported here also raise the question of the origin of positive supercoiled SSV1 DNA. This DNA was obtained from viral particles or infected cells only after amplification due to UV irradiation (28). We found recently that UV induction does indeed promote positive supercoiling, as the SSV1 DNA isolated from nonirradiated cells is much less positively supercoiled than those isolated from UV-treated cells (14). It is possible that the stress due to UV irradiation changes the topology of the SSV1 DNA by altering the balance among the transcription, the activity of the reverse gyrase, and the amount of available histonelike proteins.

The relaxed state of pGT5 could therefore more closely correspond to the chromosome topology in extreme and hyperthermophilic archaebacteria than the positive SSV1 DNA previously isolated from UV-treated cells. Preliminary results of trimethylpsoralen photobinding to chromosomal DNA of *S. shibatae* (14) indicate that this photobinding rate does not change upon γ-ray irradiation, suggesting that the chromosome is either relaxed and/or inaccessible to trimethylpsoralen cross-linking. This could be due to the presence of nucleosomelike structures, as in the case of eukaryotic chromosomes (33).

All native DNA molecules from eubacteria previously purified and analyzed exhibit a negative supercoiling (10). The pGT5 plasmid, isolated from the GE5 hyperthermophilic archaebacterium, has now been shown to exist in a relaxed state. This is so despite the presence of a reverse gyrase activity which could tend to result in a positively supercoiled DNA. Although this relaxed state would appear to be an unusual topological structure for the genome of a living organism, it can be nevertheless better reconciled with the biological activity of DNA rather than a positively supercoiled one, as SSV1 DNA. The latter contains an excess of links between the two DNA strands compared to a relaxed DNA. Thus, in a positively supercoiled DNA, all processes which require the transient opening of the double helix, such as replication or transcription, would be energetically disadvantaged. Such processes could be envisaged as energetically more favorable in the case of the relaxed pGT5 DNA from strain GE5, a hyperthermophilic archaebacterium.

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REFERENCES

1. **Auzende, J.-M., T. Urabe, and l'équipage scientifique.** 1989. Le cadre géologique d'un site hydrothermal actif: la campagne "Starmer 1" du submersible Nautile dans le bassin Nord Fidjien. C. R. Acad. Sci. Paris **302:**1787–1795.
2. **Barzilaï, R. J.** 1973. SV40: quantitative conversion of closed circular to open circular form by an ethidium bromide-restricted endonuclease. J. Mol. Biol. **74:**739–742.
3. **Bauer, W. R.** 1978. Structure and reactions of closed duplex DNA. Annu. Rev. Biophys. Bioeng. **7:**287–313.
4. **Bouthier de la Tour, C., C. Portemer, R. Huber, P. Forterre, and M. Duguet.** 1991. Reverse gyrase in thermophilic eubacteria. J. Bacteriol. **173:**3921–3923.
5. **Bouthier de la Tour, C., C. Portemer, M. Nadal, K. O. Stetter, P. Forterre, and M. Duguet.** 1990. Reverse gyrase, a hallmark of the hyperthermophilic archaebacteria. J. Bacteriol. **172:**6803–6808.
6. **Charbonnier, F.** Unpublished data.
7. **Collin, R. G., H. W. Morgan, D. R. Musgrave, and R. M. Daniel.** 1988. Distribution of reverse gyrase in representative species of eubacteria and archaebacteria. FEMS Microbiol. Lett. **55:**235–240.
8. **Crick, F. H. C.** 1976. Linking numbers and nucleosomes. Proc. Natl. Acad. Sci. USA **73:**2639–2643.
9. **Depew, R. E., and J. C. Wang.** 1975. Conformational fluctuations of DNA helix. Proc. Natl. Acad. Sci. USA **72:**4275–4279.
10. **Drlica, K.** 1992. Control of bacterial DNA supercoiling. Mol. Microbiol. **6:**425–433.
11. **Duguet, M. (Paris Sud University).** 1991. Personal communication.
12. **Erauso, G., F. Charbonnier, T. Barbeyron, P. Forterre, and D.**

Prieur. 1992. Preliminary characterization of an hyperthermophilic archaebacterium with a plasmid, isolated from a North Fiji basin hydrothermal vent. C. R. Acad. Sci. Paris **314:**387–393.

13. **Erauso, G., F. Charbonnier, T. Barbeyron, P. Forterre, and D. Prieur.** Unpublished data.
14. **Forterre, P., F. Charbonnier, E. Marguet, F. Harper, and G. Henckes.** Chromosome structure and DNA topology in extremely thermophilic archaebacteria. Biochem. Soc. Symp., in press.
15. **Forterre, P., D. Gadelle, F. Charbonnier, and M. Sioud.** 1991. DNA topology in halobacteria, p. 333–338. *In* F. Rodriguez-Valera (ed.), General and applied aspects of halophilic microorganisms, vol. 201. Plenum Press, New York.
16. **Forterre, P., G. Mirambeau, C. Jaxel, M. Nadal, and M. Duguet.** 1985. High positive supercoiling *in vitro* catalysed by an ATP and polyethylene glycol-stimulated topoisomerase from *Sulfolobus acidocaldarius*. EMBO J. **4:**2123–2128.
17. **Goldstein, E., and K. Drlica.** 1984. Regulation of bacterial DNA supercoiling: plasmid linking numbers vary with growth temperature. Proc. Natl. Acad. Sci. USA **81:**4046–4050.
18. **Grogan, D., P. Palm, and W. Zillig.** 1990. Isolate B12, which harbours a virus-like element, represents a new species of the archaebacterial genus *Sulfolobus*, *Sulfolobus shibatae*, sp. nov. Arch. Microbiol. **154:**594–599.
19. **Jaxel, C., M. Nadal, G. Mirambeau, P. Forterre, M. Takahashi, and M. Duguet.** 1989. Reverse gyrase binding to DNA alters the double helix structure and produces single-strand cleavage in the absence of ATP. EMBO J. **8:**3135–3139.
20. **Keller, W.** 1975. Determination of the number of superhelical turns in simian virus 40 DNA by gel electrophoresis. Proc. Natl. Acad. Sci. USA **72:**4876–4880.
21. **Kikuchi, A., and K. Asai.** 1984. Reverse gyrase: a topoisomerase which introduces positive superhelical turns into DNA. Nature (London) **309:**677–681.
22. **Kikuchi, A., T. Shibata, and S. Nakasu.** 1986. Reverse gyrase and DNA supercoiling in *Sulfolobus*. Syst. Appl. Microbiol. **7:**72–78.
23. **Liu, L. F., and J. C. Wang.** 1987. Supercoiling of the DNA template during transcription. Proc. Natl. Acad. Sci. USA **84:**7024–7027.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Martin, A., S. Yeats, D. Janekovic, W.-D. Reiter, W. Aicher, and W. Zillig.** 1984. SAV 1, a temperate u.v.-inducible DNA virus-like particle from the archaebacterium *Sulfolobus acidocaldarius* isolate B12. EMBO J. **3:**2165–2168.
26. **Mojica, F. J. M., F. Charbonnier, and P. Forterre.** 1992. DNA topology in halophilic archaebacteria, abstr. p87. Workshop DNA Struct. Protein Recognition. Instituto Juan March, Madrid, Spain.
27. **Nadal, M., C. Jaxel, C. Portemer, P. Forterre, G. Mirambeau, and M. Duguet.** 1988. Reverse gyrase of *Sulfolobus*: purification to homogeneity and characterization. Biochemistry **27:**9102–9108.
28. **Nadal, M., G. Mirambeau, P. Forterre, W.-D. Reiter, and M. Duguet.** 1986. Positively supercoiled DNA in a virus-like particle of an archaebacterium. Nature (London) **321:**256–258.
29. **Nakasu, S., and A. Kikuchi.** 1985. Reverse gyrase; ATP-dependent type I topoisomerase from *Sulfolobus*. EMBO J. **4:**2705–2710.
30. **Peck, L. J., and J. C. Wang.** 1983. Energetics of B-to-Z transition in DNA. Proc. Natl. Acad. Sci. USA **80:**6206–6210.
31. **Pulleyblank, D. E., M. Shure, D. Tang, J. Vinograd, and H. P. Vosberg.** 1975. Action of nicking-closing enzyme on supercoiled and nonsupercoiled closed circular DNA: formation of a Boltzmann distribution of topological isomers. Proc. Natl. Acad. Sci. USA **72:**4280–4284.
32. **Shure, M., and J. Vinograd.** 1976. The number of superhelical turns in native virion SV40 DNA and minicol DNA determined by the band counting method. Cell **8:**215–226.
33. **Sinden, R. R., J. O. Carlson, and D. E. Pettijohn.** 1980. Torsional tension in the DNA double helix measured with trimethylpsoralen in living *E. coli* cells: analogous measurements in insects and human cells. Cell **21:**773–783.
34. **Sioud, M., G. Baldacci, A. M. De Recondo, and P. Forterre.** 1988. Novobiocin induces positive supercoiling of small plasmids from halophilic archaebacteria. Nucleic Acids Res. **16:**1379–1392.
35. **Sioud, M., O. Possot, C. Elie, L. Sibold, and P. Forterre.** 1988. Coumarin and quinolone action in archaebacteria: evidence for the presence of a DNA gyrase-like enzyme. J. Bacteriol. **170:**946–953.
36. **Slesarev, A. I.** 1988. Positive supercoiling catalysed *in vitro* by ATP-dependent topoisomerase from *Desulfurococcus amylolyticus*. Eur. J. Biochem. **175:**395–399.
37. **Takahashi, M., E. Yamagushi, and T. Uchida.** 1984. Thermophilic DNA ligase: purification and properties of the enzyme from *Thermus thermophilus* HB8. J. Biol. Chem. **259:**10041–10047.
38. **Wang, J. C.** 1974. The degree of unwinding of the DNA helix by ethidium: titration of twisted PM2 DNA molecules in alkaline cesium chloride density gradients. J. Mol. Biol. **89:**783–801.
39. **Wang, J. C.** 1987. Recent studies of DNA topoisomerases. Biochim. Biophys. Acta **909:**1–9.
40. **Yeats, S., P. McWilliam, and W. Zillig.** 1982. A plasmid in the archaebacterium *Sulfolobus acidocaldarius*. EMBO J. **9:**1035–1038.
41. **Zillig, W., F. Gropp, A. Henschen, H. Neumann, P. Palm, W.-D. Reiter, M. Rettenberger, H. Schnabel, and S. Yeats.** 1986. Archaebacterial virus host systems. Syst. Appl. Microbiol. **7:**58–66.
42. **Zillig, W., S. Yeats, I. Holz, A. Böck, M. Rettenberger, F. Gropp, and G. Simon.** 1986. *Desulfurolobus ambivalens*, gen. nov., sp. nov., an autotrophic archaebacterium facultatively oxidizing or reducing sulfur. Syst. Appl. Microbiol. **8:**197–203.