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Enhancement and inhibition of DNA transcriptional activity by spermine: A marked difference between linear and circular templates

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Abstract **We compared the transcriptional activities of the circular and linear forms of short (4 kbp) and giant (106 kbp) DNA molecules in the presence of a polyamine, spermine (4+). With an increase in the spermine concentration, transcriptional activity was enhanced, followed by an inhibitory effect, and complete inhibition was observed in the sole case of long, linear templates. A difference between the transcriptional properties of circular and linear conformations is found for giant DNA molecules. These results are discussed in relation to DNA conformational transitions.**

Keywords: Higher-order structure of DNA; Transcriptional activity; Circular DNA; DNA compaction; Spermine

1. Introduction

It is generally considered that DNA biochemical functions are controlled at the sequence level by specific interactions with proteins or regulatory factors. Thus, a complex network of key–lock relations is considered to play a central role in the self-regulation of genetic activity. However, large-scale computer simulations based on the understanding of individual interactions have indicated that the reliable regulation of biological functions becomes difficult in the noisy cellular environment [1]. It is argued that switching the activity of several genes through key–lock interactions becomes unstable even for a small number of regulatory factors in the confined cellular environment, suggesting that some processes beside specific key–lock interactions play indispensable roles in the control and protection of genetic information. Structural modifications of histones and DNA such as acetylation and methylation [2,3] have been found to induce significant changes in genetic function in living cells, and it has also been shown that non-enzymatic processes that result in ordered DNA structures play a dominant role in DNA protection in starved bacteria [4]. However, our understanding of the importance and significance of the change or transition in the DNA higher-order structure induced by non-specific environmental chemical factors seems to be at a primitive stage, and the pertinent physical parameters of this process have not yet been identified.

The higher-order structure of DNA chains can be controlled in physiological-mimetic conditions by modifying the solution environment, which results in DNA collapse from an unfolded, random-coil state to a folded, compact and ordered state as reviewed in [5]. This phenomenon has been interpreted in terms of DNA condensation or compaction, and has been extensively studied with regard to its significance in a variety of fields, ranging from polymer physics to molecular biology, and its potential applications in gene therapy [6]. With respect to the relationship between the higher-order structure of DNA and its transcriptional activity, apparently conflicting effects have been reported in past studies. Experiments performed at the level of an ensemble of molecules as well as at the single-chain level have clearly indicated that a promoter located near the center of a long linear DNA (lambda ZAPII, 40 kbp) is not accessible to RNA polymerase in the compact state, i.e., compaction is expected to prevent the binding/sliding of RNA polymerase to DNA [7,8]. On the other hand, it was reported that pBR322, a short circular DNA (4 kbp), was still transcriptionally active after condensation induced by spermidine [9].

Compaction by multivalent polycations is achieved through almost complete neutralization of the negative charge along the DNA backbone [10,11]. Giant linear DNAs larger than several kilo-base pairs exhibit an all-or-none transition between elongated coil and folded compact states [12,13]. On the other hand, fluorescent microscopic observations recently revealed that the folding of circular DNA on the order of several kilo-base pairs occurs as a continuous transition even at the individual molecule level, and results in a rather loosely collapsed state [14,15]. In this study, we performed transcription experiments on circular and linear versions of short and long DNA and found that circularity prevents the complete inhibition of transcriptional activity with compaction, and that the difference between the two conformations increases with DNA length. We also confirmed the previously reported promotion of transcription at lower spermine concentrations.

2. Materials and methods

2.1. DNA substrates

Circular pBR322 plasmid DNA (4361 bp) was purchased from Wako, Nippon Gene. Linearized pBR322 DNA was obtained from Toyobo after treatment by the restriction enzyme Pvu II. Circular BAC DNA (106 kbp) was prepared according to the protocol

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Abbreviation: bp, base pairs

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described in [16], and its linear form was prepared by treatment with the restriction enzyme SalI followed by phenol-extraction and ethanol-precipitation. The restriction sites were chosen far from transcription promoters to avoid affecting transcriptional properties.

2.2. Transcription and detection of RNA products

ATP, CTP and GTP were purchased from Roche Diagnostics, and stock solutions of 1 M Tris–HCl buffer, 1 M $MgCl_2$, 5 M NaCl, and spermine tetrachloride were obtained from Nakalai Tesque, Kyoto. UTP gamma-AmNS (absorption/emission maxima ~330/463 nm; Molecular Probes), in which the fluorescent aminonaphthalenesulfonate (AmNS) is attached to the terminal phosphate of UTP, was used to monitor transcriptional activity. The DNA template concentrations during transcription were 0.96 μg/100 μl (circular) and 0.90 μg/100 μl (linear) for pBR322 DNA, and 0.75 μg/100 μl (circular) and 0.65 μg/100 μl (linear) for BAC7/8-α2 DNA. Transcription was performed in 100-μl reaction solutions under nuclease-free conditions, containing 10 mM Tris–HCl buffer (pH 7.6), 100 mM KCl, 5 mM $MgCl_2$, 0.1 mM ATP, CTP and GTP, 0.01 mM UTP gamma-AmNS, 2 mM dithiothreitol, and various concentrations (0–1400 μM) of spermine. After 10 min of pre-incubation to reach equilibrium, two units of *E. coli* RNA polymerase holoenzyme (Epicenter) were added and transcription was performed for 2 h at 37 °C. For kinetic measurements, 40-μl aliquots were removed at regular intervals from tubes with an original volume of 250 μl. The reaction was stopped by the addition of 60 μl of 50 mM EDTA. Transcription products were detected by measuring the sample fluorescence intensity at 475 nm using a JASCO FP-750 spectro-fluorometer.

2.3. Fluorescence microscopic observations

One micromolar in base pair units of linear BAC DNA in transcription buffer was stained with 1 μM fluorescent dye DAPI (Wako Pure Chemical Industries, Osaka, Japan). The samples were then loaded onto glass cover slips washed with ethanol, and observed using an inverted fluorescence microscope (Olympus IX70) equipped with a 100× oil-immersed objective lens and a Hamamatsu Photonics EBCCD camera.

3. Results and discussion

Transcriptional activity was measured in arbitrary units though the fluorescence intensity resulting from the separation of the AmNS group from UTP-gamma-AmNS during RNA polymerization [17]. It has been confirmed that there is no significant effect of spermine on RNA conformation under the experimental conditions adopted in the present study. To assess the effect of the addition of spermine on transcription, we plotted relative transcriptional activity (Fig. 1), defined as the ratio of RNA products at a given spermine concentration to the amount of RNA products in the absence of spermine. The actual amounts of transcription products are shown on Fig. 2. When the spermine concentration reached a certain value, a decrease in transcriptional activity associated with the folding transition of DNA was observed in all cases, and this inhibitory effect was particularly steep for linear templates. Complete inhibition of transcriptional activity was achieved in the sole case of long and linear templates, while some remaining transcriptional activity was detected for other DNAs in the explored range of spermine concentrations. Circular templates in particular showed a soft transition with a higher level of surviving activity. At low spermine concentrations, around [spermine] = 500 μM, we observed an enhancement of transcription that could be attributed to the decrease of DNA negative charge before the conformational transition

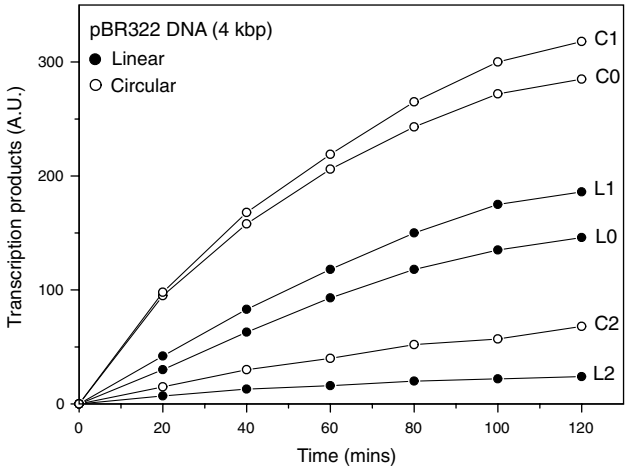

Fig. 2. Transcription kinetics of circular and linear pBR322 DNA in the absence of spermine (C0, L0), with 400 μM spermine corresponding to the enhancement of transcriptional activity prior to compaction (C1, L1), and 1200 μM spermine corresponding to compacted DNA (C2, L2).

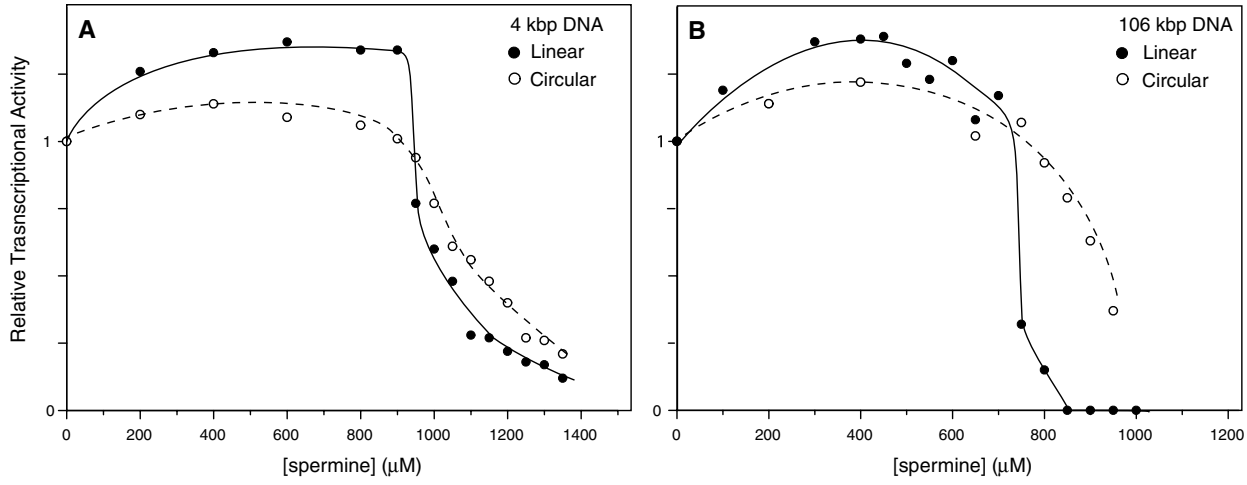

Fig. 1. Typical profiles of relative transcriptional activity of pBR322 DNA (A) and BAC DNA (B) with respect to spermine concentration for the linear (full line) and circular (broken line) conformations of each DNA. The activities at 0 μM spermine are taken as unity.

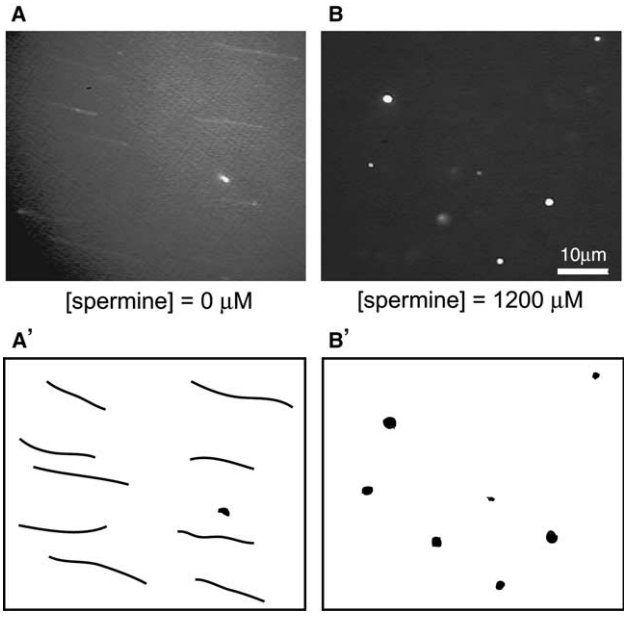

Fig. 3. Fluorescence microscopic images of linear 106-kbp DNA adsorbed on a glass surface and corresponding schematic representations. In the absence of spermine (A, A′), coiled molecules could be stretched on the surface, whereas at 1200 μM spermine (B, B′) only single compact molecules or multiple molecules aggregates were observed. Similar results were observed for pBR322 DNA.

[11], which thereby reduced the energy required for binding between negatively charged RNA polymerase and DNA [18]. This enhancement was stronger in the case of linear chains, suggesting that the possible modification of DNA chain rigidity though spermine weak binding may also facilitate transcription. To evaluate the quantity of RNA synthesized and the time-dependence of the reaction, we performed kinetic measurements at different spermine concentrations, as shown in Fig. 2. Standard transcription profiles with finite amount of substrate were observed with an initial linear velocity, progressing towards a plateau. Under the same experimental conditions, circular templates yielded about two times more RNA products than did linear templates, for all spermine concentrations. This suggests that superhelicity may facilitate interaction between DNA and binding proteins, as supported by a previous report that diffusion of the endonuclease *Eco*RV is more efficient for supercoiled than relaxed DNA [19].

To confirm that the inhibition of transcription was due to the transition in the higher-order structure of DNA, we checked the DNA conformation by fluorescence microscopy (Fig. 3). In the absence of spermine and at a low spermine concentration, we observed stretched molecules corresponding to DNA in a swollen state, while at the high spermine concentrations corresponding to inhibition no stretched molecules were found. Instead, we observed single compact molecules or aggregates of compact molecules, indicating a significant change in the DNA conformation. The partial aggregation of DNA condensates increases with the spermine concentration and may be responsible for the decrease in transcriptional activity following the folding transition.

We now present a few arguments in addition to the direct observations that indicate a correlation between DNA conformation and transcriptional activity. If short DNA of around 150 bp is condensed by a polyamine, a fraction will remain in the supernatant [20]. The efficiency of compaction is probably related to the DNA length, and although the efficiency of condensation in the case of ~4 kbp DNA is not known, the difference in activity for linear and circular templates suggests that the remaining activity around the spermine concentration corresponding to condensation results from the slight activity of the compacted DNA. The transition profiles correspond to the expected physical properties during compaction. When a polymer's length increases, the coil-globule transition adopts an on/off switching nature. The possibility that DNA undergoes a discrete coil-globule transition is a specific property that appears only when the chain can be regarded as a semi-flexible polymer, i.e., when its length exceeds ~10 times its Kuhn length (300 bp or 100 nm). According to this theoretical consideration, pBR322 DNA is not long enough for an on/off conformational transition, which prevents complete compaction, and therefore allows some degree of transcription for linear pBR322 DNA. Since RNA polymerization requires the binding of polymerase at a specific sequence and its diffusion along/around DNA [21,22], the transcriptional activity of condensates should depend on their shape and local density. Measurements of Brownian motion indicated a small difference in density between elongated and compact states for a 12.5 kbp circular DNA [14], whereas the change in density for linear giant DNA is on the order of 10^3–10^4. Compaction in the case of circular DNA may consequently be loose enough so that RNA polymerase can access target DNA sequences. Other studies using electron microscopy and AFM have indicated that circular DNA that is compacted by polyamine or neutral polymer adopts a toroid or rodlike shape [9,23]. However, it is difficult to confirm whether or not the DNA conformation remains essentially the same during transcription, especially since RNA molecules can unfold compact DNA [24]. It may be useful to characterize the role of spermine in the cell cycle with respect to its ability to alter DNA-protein interactions [18].

The different behaviors that we observed support the notion that complete or partial inhibition of transcription results from condensation in cooperation with the different transition properties of circular and linear DNA. Thus, different DNA morphologies and lengths lead to different transcriptional activities in compact DNA. The differences between compact states imply that a circular structure is important for the retention of transcriptional activity, and that modification of the superhelicity in long DNA enables the switching of gene activity though conformational transitions. Since long genetic polymers in vivo may be unexpectedly condensed in response to slight changes in the chemical composition of the surrounding environment, the present results also imply that superhelicity in DNA may protect biophysical activities from accidental condensation by basic materials. It should be noted that during gene expression in eukaryotes, the partial unfolding of DNA from its tight packing around histones results in the opening of a transcription loop with two ends, which is topologically equivalent to a closed circular structure. DNA length also appears to be a critical parameter involved in the control of transcription. In addition to chemical controls at the nanometer scale, conformational changes in the higher-order structure of DNA at the micrometer scale or above the size of several tens of kilo-base pairs may contribute to the fine control or tuning of biochemical activity.

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References

[1] Paulsson, J. (2004) Summing up the noise in gene networks. Nature 427, 415–418.
[2] Pennisi, E. (1997) Opening the way to gene activity. Science 275, 155–157.
[3] Eberharter, A. and Becker, P.B. (2002) Histone acetylation: a switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. EMBO Rep. 3, 224–229.
[4] Frienkel-Krispin, D., Levin-Zaidman, S., Shimoni, E., Wolf, S.G., Wachtel, E.J., Arad, T., Finkel, S.E., Kolter, R. and Minsky, A. (2001) Regulated phase transitions of bacterial chromatin: a non-enzymatic pathway for generic DNA protection. EMBO J. 20, 1184–1191.
[5] Bloomfield, V.A. (1996) DNA condensation. Curr. Opin. Struct. Biol. 6, 334–341.
[6] Behr, J.-P. (1994) Gene-transfer with synthetic cationic amphiphiles – prospects for gene-therapy. Bioconjug. Chem. 5, 382–389.
[7] Tsumoto, K., Luckel, F. and Yoshikawa, K. (2003) Giant DNA molecules exhibit on/off switching of transcriptional activity through conformational transition. Biophys. Chem. 106, 23–29.
[8] Yamada, A., Kubo, K., Nakai, T., Yoshikawa, K. and Tsumoto, K. (2005) All-or-none switching of transcriptional activity on single DNA molecules caused by a discrete conformational transition. Appl. Phys. Lett. 86, 223901.
[9] Baeza, I., Aguilar, L., Santiago, R., Ibáñez, M. and Wong, C. (1997) Electron microscopy and biological properties of pBR322 DNA condensed with the trivalent cations spermidine and hexamine cobalt (III). Rev. Lat.-Am. Microbiol. 39, 47–56.
[10] Wilson, R.W. and Bloomfield, V.A. (1979) Counterion-induced condensation of deoxyribonucleic acid. A light scattering study. Biochemistry 18, 2192–2196.
[11] Yamasaki, Y., Teramoto, Y. and Yoshikawa, K. (2001) Disappearance of the negative charge in giant DNA with a folding transition. Biophys. J. 80, 2823–2832.
[12] Yoshikawa, K., Takahashi, M., Vasilevskaya, V.V. and Khokhlov, A.R. (1996) Large discrete transition in a single DNA molecule appears continuous in the ensemble. Phys. Rev. Lett. 76, 3029–3031.
[13] Mel'nikov, S.M., Sergeyev, V.G. and Yoshikawa, K. (1995) Transition of double-stranded DNA chains between random coil and compact globule states induced by cooperative binding of cationic surfactant. J. Am. Chem. Soc. 117, 9951–9956.
[14] Sato, Y., Hamada, T., Kubo, K., Yamada, A., Kishida, T., Mazda, O. and Yoshikawa, K. (2005) Folding transition into a loosely collapsed state in plasmid DNA as revealed by single-molecule observation. FEBS Lett. 579 (14), 3095–3099.
[15] Velichko, Y.S., Yoshikawa, K. and Khokhlov, A.R. (1999) Effect of twisting on the behavior of a double-stranded polymer chain: a Monte-Carlo simulation. J. Chem. Phys. 111, 9424.
[16] Hizume, K., Yoshimura, S. and Takeyasu, K. (2004) Atomic force microscopy demonstrates a critical role of DNA superhelicity in nucleosome dynamics. Cell Biochem. Biophys. 40, 249–262.
[17] Dunkak, K.S., Otto, M.R. and Beechem, J.M. (1996) Real-time fluorescence assay system for gene transcription: simultaneous observation of protein/DNA binding, localized DNA melting, and mRNA production. Anal. Biochem. 243, 234–244.
[18] Thomas, T. and Thomas, T.J. (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol. Life Sci. 58, 244–258.
[19] Gowers, D.M. and Halford, S.E. (2003) Protein motion from non-specific to specific DNA by three-dimensional routes aided by supercoiling. EMBO J. 22, 1410–1418.
[20] Pelta, J., Livolant, F. and Sikorav, J.L. (1995) DNA aggregation induced by polyamines and cobalthexamine. J. Biol. Chem. 271, 5656–5662.
[21] Bustamante, C., Guthold, M., Zhu, X. and Yang, G. (1999) Facilitated target location on DNA by individual *Escherichia coli* RNA polymerase molecules observed with the scanning force microscope operating in liquid. J. Biol. Chem. 274, 16665–16668.
[22] Halford, S.E. and Marko, J.F. (2004) How do site-specific DNA-binding proteins find their targets?. Nucleic Acids Res. 32, 3040–3052.
[23] Martin, A.L., Davies, M.C., Rackstraw, B.J., Roberts, C.J., Stolnik, S., Tendler, S.J.B. and Williams, P.M. (2000) Observation of DNA–polymer condensate formation in real time at a molecular level. FEBS Lett. 480, 106–112.
[24] Tsumoto, K. and Yoshikawa, K. (1999) RNA switches the higher-order structure of DNA. Biophys. Chem. 82, 1–8.