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# Giant DNA molecules exhibit on/off switching of transcriptional activity through conformational transition

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**Abstract**

We found that the transcriptional activity of large DNAs (40 kbp) can be completely inhibited by adding condensing agents, spermine and poly(ethylene glycol), whereas under the same conditions short fragments (140 bp) still show active transcription. Fluorescence microscopic observations of large DNAs revealed clear correlation between the higher-order structure of templates and their transcriptional activity. The steep decrease in transcriptional activity leading to complete inhibition, or on/off switching, is interpreted in terms of conformational transition of the ensemble of DNA molecules.


*Keywords:* Transcriptional activity; DNA condensation; Higher-order structure of DNA; Spermine; Poly(ethylene glycol); Fluorescence microscopy

## 1. Introduction

Genetic information in living cells is generally embedded in long DNA molecules. *Escherichia coli*, which is only 1 μm in diameter, contains genomic DNA that is 1 mm long. Human genomic DNA, which has a total length of approximately 2 m, is packed within the narrow space of the nucleus. It is well known that conformational changes in the higher-order structure of chromosomal DNA, such as compaction and loosening, are strongly correlated with gene activities in both eukaryotic [1] and prokaryotic cells [2], implying that the manner of packing and unfolding may be related to the regulation of genetic activity. Acetylation/deacetylation and methylation of chromatin, together with changes in the composition of interacting proteins, have been suggested to be the main cause of changes in the higher-order structure of DNA, which lead to gene regulation [3,4].

The phenomenon known as 'DNA condensation' can be achieved under both non-physiological and physiological conditions, and is of particular interest as an in vitro model system for examining such biological properties [5–7]. Various kinds of condensing agents, such as polycations (polyamine, cobalt hexamine), neutral polymers

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[poly(ethylene glycol)], alcohol, etc. [5–8], have been examined and are known to cause DNA collapse into an ordered, compact state. In contrast to the previous notion that DNA condensation was a diffuse transition [9], recent observations of single DNA molecules by fluorescence microscopy revealed that long DNA chains individually undergo conformational transition from an unfolded, random-coil state to a compact, folded state in an all-or-none, or biphasic, manner [10,11]. This on/off nature at the single molecular level is considered to be general for double-stranded DNAs larger than ~10 kbp, while short DNA fragments (~400 bp) cannot undergo such folding transition [5–7].

It has previously been reported that condensing agents affect the biochemical reactivity of DNA (e.g. digestion [12], ligation [13] and transcription [14]), with activation and inhibition occurring at certain concentrations. Intermolecular aggregation of DNA can occur at high agent concentrations, leading to undesirable inhibition of enzymatic activity. Therefore, experimental conditions are usually adjusted to prevent DNA condensation/precipitation, and there have only been a few studies on the correlation between the higher-order structure of DNA and reactivity. For example, it has been shown that polyamine can induce the activation of topoisomerases [15] and RNA polymerases [16] accompanied by 'DNA condensation', where the term 'DNA condensation' is limited to multiple-chain events, possibly including single-chain compaction [6]. Consequently, the biochemical reactivity of DNA has not been satisfactorily understood in terms of the conformational 'switching' of individual large DNA molecules.

In this study, we examined how changes in the higher-order structure affect the transcriptional activity of a long linear DNA, Lambda ZAP II (40 kbp), as compared to a short DNA template (140 bp) with the same promoter. We used two different condensing agents, spermine and poly(ethylene glycol) (PEG), which are well known to have opposite properties: condensation induced by polycations, such as spermine (4+), is promoted by a temperature increase and inhibited by the addition of salt [17,18], while opposite trends are observed with neutral polymers, such as PEG [19]. We show that a drastic inhibition of transcriptional activity occurs in the sole case of large DNA templates, and we propose an interpretation based on the all-or-none folding transition of long DNA chains.

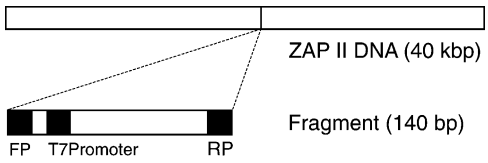


Fig. 1. Schematic representation of short DNA fragments with the corresponding Lambda ZAP II DNA template.

## 2. Materials and methods

### 2.1. Transcription

Lambda ZAP II DNA (recombinant DNA of bacteriophage lambda origin; Stratagene, La Jolla, CA) including a T7 promoter was used as a template for transcription by T7 RNA polymerase (T7 RNAP; Invitrogen, Gaithersburg, MD). The nucleotides ATP, CTP, GTP and UTP (NTPs) were purchased from Roche Diagnostics (Basel, Switzerland), and dithiothreitol (DTT) was provided with the polymerase kit. Stock solutions of 1 M Tris–HCl buffer, 1 M $MgCl_2$, 5 M NaCl, spermine tetrachloride and poly(ethylene glycol) 20 000 were obtained from Nacalai Tesque (Kyoto, Japan). During sample preparation, precautions were taken to prevent contamination with nucleases. For the control experiment, 140-bp fragments including a T7 promoter were prepared by polymerase chain reaction (Takara Taq Hot Start; Takara Shuzo, Otsu, Japan) using Lambda ZAP II DNA as a template and a set of synthesized primers (forward TAAAA CGACG GCCAG TGAGC; reverse CCGCT CTAGA ACTAG TGGAT), as illustrated by the scheme in Fig. 1. After purification, the monodispersity of PCR products was confirmed by electrophoresis on agarose gel.

Transcription was performed in 50-μl reaction solutions containing 10 mM Tris–HCl buffer (pH 7.6), 100 mM NaCl, 5 mM $MgCl_2$, 0.5 mM NTPs

and 5 mM DTT. The concentration of condensing agents was 0–800 μM for spermine or 0–160 mg/ml for PEG, while the concentration of large or short DNA templates was fixed at 2 μg/ml (~3 μM of bp) in all of the experiments. After adding 50 U of T7 RNAP, the solution was incubated at 37 °C for 1–1.5 h. The reaction was stopped by adding Tris-saturated phenol. RNA products and DNA templates were recovered by ethanol precipitation, dried and rehydrated with distilled water. The DNA templates were then digested with 1 U of deoxyribonuclease (Wako Nippon Gene, Tokyo, Japan) at 37 °C for 1.5 h.

### 2.2. Measurement of RNA concentration

The samples were stained by adding an equal volume of 200-fold-diluted RiboGreen (fluorescent dye for RNA quantitation; Molecular Probes, Eugene, OR; maximal excitation at 500 nm) in Tris–EDTA (TE) buffer, and the fluorescence intensity was measured using an FP-750 spectrofluorometer (Jasco, Hachioji, Japan). The spectrum intensity was read in arbitrary units at 525 nm.

### 2.3. Fluorescence microscopic observation

DNA molecules were stained with fluorescent dyes 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Wako Pure Chemical Industries, Osaka, Japan) or YOYO-1 (Molecular Probes) as mentioned in detail in the following section. The samples were loaded onto clean glass cover slips, and the objects adsorbed on the glass surface were observed with an inverted fluorescence microscope (Zeiss Axiovert 135 TV) equipped with a 100× oil-immersed objective lens and a Hamamatsu Photonics high-sensitivity SIT TV camera.

## 3. Results

We performed experiments on the transcriptional activity of large DNAs and short fragments in the presence of condensing agents, spermine and PEG, under similar experimental conditions. Transcriptional activity was evaluated in terms of fluorescence intensity, which reflects the total amount of RNA product recovered from the reaction solution. Fig. 2a,b show the changes in the transcriptional activity of long linear DNA templates (Lambda ZAP II, 40 kbp) with the addition of condensing agents, spermine and PEG, respectively. Transcriptional activity abruptly decreased when the concentration of the condensing agents reached a critical value; for both spermine and PEG, complete inhibition was observed above a certain concentration. Below the critical concentration of spermine, the activity was maximal at approximately 200 μM (Fig. 2a). While the activation of transcriptional activity at a low spermine concentration has been reported previously, abrupt inhibition subsequent to a conformational change had not been noted [20].

Fig. 2c,d shows the transcriptional activity of short DNA templates (140 bp), where the molar ratio of DNA phosphates to condensing agents was the same as it was for experiments with large DNAs. The changes in transcription at low concentrations were similar for long and short DNA templates, but in contrast to the steep inhibition observed in the case of large DNAs, short fragments showed a gradual decrease in the presence of excess condensing reagents without reaching complete inhibition.

Fig. 3a shows typical fluorescence microscopic images of large DNA templates in the presence of spermine. In order to avoid time-dependent changes in the solution environment due to transcriptional reaction during the observations of DNA conformation, T7 RNAP was not added to the samples. DNA conformation was found to be significantly different below or above a critical concentration, [spermine] ≈ 420 μM, corresponding to the abrupt decrease in transcription. At [spermine] = 400 μM, most DNA molecules dispersed on the glass surface in a swollen form. In contrast, aggregates with multiple compact DNA chains were observed at [spermine] = 450 μM. While single compact DNA molecules are usually observable at dilute DNA concentration, the high DNA concentration needed here for a high yield of RNA products was so that partial aggregation followed the compaction of individual DNA molecules. Short fragments, on the other hand, did not form large aggregates at any spermine concentration (data not shown).

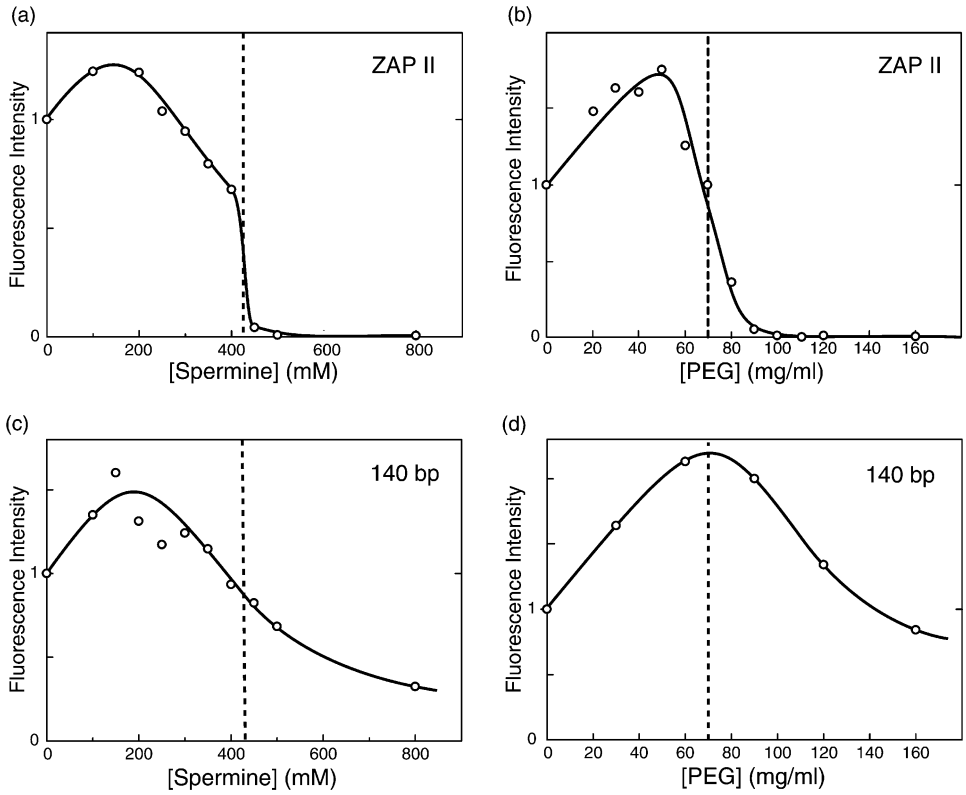


Fig. 2. Transcriptional activity vs. concentration of condensing agents for Lambda ZAP II DNA (*top*) and 140-bp fragments (*bottom*) using (a,c) spermine and (b,d) PEG. The activity in each graph is normalized to that in the absence of condensing agents. The vertical broken line indicates the position near the transition region for changes in the DNA conformation, as determined by fluorescence microscopic observation (see Fig. 3).

A similar experimental trend was observed with PEG, with elongated and compact states coexisting between 60 and 80 mg/ml. To overview the conformational change tendency of individual large DNAs without multiple aggregations, we adopted a lower concentration of a slightly larger DNA (bacteriophage lambda DNA, 48 kbp) with 100 mM NaCl, close to the ionic strength in transcriptional conditions. Fig. 3b shows fluorescence images of compact DNA molecules at a higher PEG concentration, [PEG] = 200 mg/ml. All these results suggest that DNA chains undergo a folding transition into a compact state above a certain condensing agent concentration, leading to the complete inhibition of transcription.

## 4. Discussion

The above results indicate that the change in transcriptional activity with the addition of condensing agents is highly dependent on the size of the DNA template. The steep transitions in large DNAs around the critical concentrations, [spermine] ≈ 420 μM and [PEG] ≈ 70 mg/ml, are remarkable. On the other hand, short DNAs still show transcriptional activity even above the critical concentrations of the condensing agents (Fig. 2c,d). The length of the short DNAs, 140 bp, is of the order of the persistence length (150 bp; 50 nm) of double-stranded DNA [5], suggesting that short templates behave as rigid rods. Consequently,

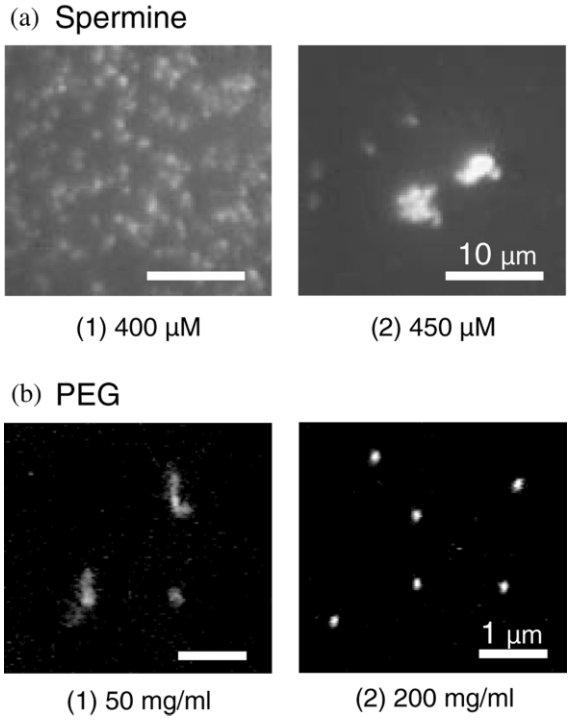


Fig. 3. Fluorescence microscopic images of large DNA molecules adsorbed on the glass surface. (a) Lambda ZAP II DNA observed under the transcription conditions in solution excluding T7 RNA polymerase, stained with 0.3 μM DAPI: swollen state with 400 μM spermine (a1) and compact/aggregated states with 450 μM spermine (a2). (b) Effect of PEG on the conformation of 0.1 μM bp lambda phage DNA (48 kbp; Takara Shuzo) stained with 0.33 μM YOYO-1 in a solution of 100 mM NaCl: unfolded state at 50 mg/ml PEG (b1) and compact state at 200 mg/ml PEG (b2).

a folding transition into a compact state cannot occur. However, consistent with the present results, short fragments can partially form aggregates made up of several molecules at high concentrations of DNA or condensing agent [21]. Lambda ZAP II DNA, which is approximately 270-fold longer than the persistence length, can undergo an all-or-none transition into a compact state. In DNA condensation/compaction, the folding of large DNA chains into an ordered structure is accompanied by a significant increase in the segment density, typically of four–five orders of magnitude [8,22]. This may exclude proteins/enzymes that require binding and/or sliding on DNA chains, leading to complete inhibition of the reaction. Recently, it has been demonstrated that the activity of a restriction enzyme, *Apa*LI, on lambda phage DNA (48 kbp) is completely inhibited by the addition of spermine at the critical concentration for inducing the folding transition of DNA [23].

An increase in transcriptional activity was similarly observed at low condensing agent concentrations, for both long and short DNA templates. Since an increase in NaCl concentration from 0 to 100 mM also linearly increases Lambda ZAP II DNA transcriptional activity without causing DNA condensation, this promotion of transcription may not be directly correlated to the global compaction of large linear DNAs. Further careful examinations are necessary to clarify the actual causes of transcriptional promotion, as well as its gradual decrease below the critical concentration in the case of spermine. In the case of PEG, the apparent promotion is attributable, at least partly, to the remaining presence of viscous PEG during sample handling for RNA product recovery.

Based on the above results and discussion, the length of DNA templates is found to be a primary factor in the complete inhibition of transcriptional activity. The remarkable effect of the conformational transition on transcriptional activity implies that the largeness of DNA chains, such as genomic DNAs, might ensure complete inhibition of transcription, or silencing of gene activity, in response to changes in the solution environment. Complete inhibition of the expression of certain genes is essential for development and differentiation [1]. Since it can be expected that chromosomal DNAs retain the dynamic properties of long polymer chains in vivo, even if they take a chromatin form in eukaryotic cells [24], it is worth discussing the plausible biological significance of the large size of DNAs.

Here, we would like to provide a simple argument based on the apparent ‘cooperativity’ of the inhibiting phenomena, instead of considering detailed mechanisms. According to the standard biochemical model, steep changes in biochemical reactions can usually be interpreted using the following equation:

$$v \propto \frac{1}{1+x^n} \tag{1}$$

where the parameter $n$ indicates the degree of cooperativity, and the reaction rate $v$ is phenomenologically given as a function of the concentration $x$ of chemical species in a reaction. To reproduce the steep profiles of transcriptional inhibition as in Fig. 2, the cooperativity for the interaction among T7 RNAP, DNA and NTPs should have a quite unrealistic, unusually excessive value. Since long and short DNA templates show different behaviors, it is clear that the interpretation based on this concept of cooperativity is not appropriate here. Instead, the polymer nature of long templates, i.e. their ability to undergo an on/off transition in their higher-order structure, can explain the steep change in transcriptional activity exhibited solely by long templates. The results in the present study clearly show that the steep inhibitions of transcriptional activity correspond to a marked conformational transition. Therefore, by considering changes in the populations of the folded and unfolded states depending on the solution environment at the level of individual molecules, it is possible to interpret the apparent high 'cooperativity' observed in the case of large DNA templates [25,26]. In this context, the relative population $P_u$ of unfolded DNA ($P_u = 1$, all molecules are unfolded; $P_u = 0$, all are folded) is expressed as a function of the condensing agent concentration (molar units) as:

$$\ln\frac{1-P_u}{P_u} = m\ln x + C \quad (2)$$

where $m$ is the effective number of condensing agent molecules in the folding transition of a single large DNA and $C$ is a constant [26]. As for large DNA chains, $m$ can be several 10s. If we assume that the reaction rate $v$ is approximately proportional to the relative population of unfolded DNA, i.e. DNA in an active form, Eq. (2) becomes:

$$\ln\frac{v_0 - v}{v} \approx m\ln x + C \quad (3)$$

where $v_0$ is the reaction rate in the absence of an inhibitory effect. In a semi-quantitative manner, at least, Eq. (3) can explain the drastic inhibitory change in transcriptional activity. Using this mechanism, we can also explain the marked difference between large and short DNAs.

We have demonstrated here that the on/off switching framework of the higher-order structure can be introduced to interpret 'cooperative-like' biochemical reactivity of DNA templates of a sufficient length. The conformational transition properties of long DNA depend on the composition of the solution environment, particularly histones [27], polyamines, ATP [28], RNA [29] and low-valency salts [18,30]. An appropriate, even slight, change in such environments might therefore control the accessibility of regulating biomacromolecules effectively to genes located on long DNAs [31]. Although the higher-order structure of DNA has been regarded as one of the most important factors in gene regulation, previous reports have described apparently contradictory correlations between DNA conformation and biochemical reactivity [15,16,23,32]. It may be useful to characterize the significance of the unfolded and folded states of large DNA with regard to biochemical functions.

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