THE INFLUENCE OF DNA GYRASE ON THE TRANSCRIPTION OF LINEAR DNA IN VITRO

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1. Introduction

DNA gyrase could negatively supercoil DNA in an ATP-driven reaction. Numerous data obtained both in vivo and in vitro show the depression of such vital processes as replication and transcription and of integrative recombination by the inhibitors of DNA gyrase [1]. These data prove that for proper functioning the DNA should be supercoiled. However, some observations do not fit this picture: DNA gyrase inhibitors hinder the replication and transcription of linear DNA molecules, as shown in vivo for T7 phage DNA [2] and in vitro for the ColE1 linear DNA [3]. To explain these one could suppose that DNA gyrase is capable of supercoiling linear DNA although it is not possible to detect this (probably transient) supercoiling by commonly accepted methods. Therefore, we have tested this possibility by making use of an increased transcriptional level of supercoiled DNA as compared with relaxed DNA [1,4]. We show here that DNA gyrase markedly stimulates the transcription of linear DNA by purified RNA polymerase, the stimulation being connected with the DNA gyrase supercoiling activity.

2. Experimental

RNA polymerase was purified from *Escherichia coli* cells by the method of [5] as described in [6]. RNA synthesis was assayed by incubating RNA polymerase with DNA for 15 min at 30°C in 50 μl of 50 mM KCl, 10 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.5), 2 mM ATP, 0.5 mM GTP and CTP, 0.025 mM UTP, 1–2 μCi [^{3}H]UTP, 1 mM EDTA, 1 mM DTT, 4 mM K-phosphate, 4% glycerol, 400 μg bovine serum albumin/ml. It was checked separately that DNA gyrase retained its supercoiling ability under these conditions. The RNA synthesized was trichloroacetic acid-precipitated and collected on nitrocellulose membrane filters. The radioactivity was quantitated in a toluene-based scintillator.

DNA gyrase was purified by a modification of the method in [7]. The activity of the enzyme was assayed as follows: 0.6–3 μg DNA gyrase was incubated for 60 min at 25°C with 0.2 μg relaxed ColE1 DNA in 25 μl 35 mM Tris–HCl (pH 7.5), 5 mM DTT, 6 mM $MgCl_2$, 1.8 mM spermidine, 360 μg bovine serum albumin/ml, 1.4 mM ATP, 9 μg/ml tRNA [8]. The reaction was stopped by adding 6 μl 5% SDS + 125 mM EDTA, and DNA samples were electrophoresed in 1% agarose gel.

Supercoiled and relaxed ColE1 DNA were kindly gifted by Dr E. S. Bogdanova (this department). DNA was relaxed by topoisomerase I from Ehrlich ascites tumor cells [9]. T7 and λ phage DNA were obtained by phenol extraction of purified phage preparations.

3. Results and discussion

Table 1 shows that DNA gyrase appreciably stimulates the transcription of linear λ phage DNA by a purified RNA polymerase. The qualitative effect is obvious, however the extent of stimulation could vary in different experiments for unknown reasons. The supercoiling activity of DNA gyrase is required for the stimulation of transcription since the inhibitor of this activity, novobiocin, eliminates the stimulating effect. Novobiocin does not influence transcription in the absence of DNA gyrase (not shown).

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Table 1
The influence of DNA gyrase on the transcription of λ phage DNA

Enzyme	[^{3}H]UMP incorporation into RNA							
	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	cpm	%	cpm	%	cpm	%	cpm	%
RNA polymerase	26 887	100	21 889	100	53 528	100	45 020	100
DNA gyrase	809	3	1366	6	3501	6	–	–
RNA polymerase + DNA gyrase	69 128	257	36 740	167	84 580	158	72 286	160
+ novobiocin	–	–	20 859	95	57 520	107	–	–

Assay mixture contains 0.66 μg λ DNA, 1 μg RNA polymerase, 1.9 μg DNA gyrase, 0.5 μg novobiocin

The results in table 2 show that, in contrast with λ DNA, DNA gyrase has no effect on the transcription of T7 DNA by a purified RNA polymerase. So, DNA gyrase action is template-specific.

DNA supercoiling appreciably stimulates transcription. Hence, our results suggest the supercoiling of linear DNA molecules. How can this supercoiling be explained? One possible explanation is that the constant winding of DNA by DNA gyrase could lead to some equilibrium superhelix density, its value being dependent on the respective speeds of the DNA gyrase action and the spontaneous unwinding of the DNA free ends. It has been supposed that for such an effect to be significant the unwinding of free DNA ends should be somehow hindered [3]. Our results show that if the 'equilibrium' explanation is valid the rate of unwinding of free DNA ends would be sufficiently small to allow the supercoiling of DNA by the constantly acting DNA gyrase. This explanation assumes the highest density of supercoiling in the middle of the molecule and almost complete relaxation in the end regions, in accordance with our observation that DNA gyrase stimulates the transcription of λ DNA with a central location of major promoters but not that of T7 DNA with promoters lying at the very end of the molecule.

Another possible mechanism of linear DNA supercoiling presupposes a mutual fixation of the molecule ends. Such a fixation could occur on the membrane or some other cellular components [2]. We have, however, observed the stimulation in a purified in vitro system which component, given the assumption in hand, should be capable of sticking together distant segments of the DNA molecule. Although such a component could be a minor impurity, the DNA gyrase itself may be responsible for the process. The recent discovery of the sign-invertion mechanism of the DNA gyrase action makes this suggestion plausible [10]: to pass one DNA segment through the double-stranded break in another segment, DNA gyrase

Table 2
The influence of DNA gyrase on the transcription of T7 DNA

Enzyme	[^{3}H]UMP incorporation into RNA					
	Exp. 1		Exp. 2		Exp. 3	
	cpm	%	cpm	%	cpm	%
RNA polymerase	64 561	100	35 780	100	75 232	100
DNA gyrase	4813	7	2978	8	468	0.5
RNA polymerase + DNA gyrase	74 210	114	34 675	96	60 096	94

Assay mixture contains 0.5 μg T7 DNA, 2 μg RNA polymerase, 1.9 μg DNA gyrase

should mutually fix both segments while another DNA gyrase molecule can supercoil the thus formed topological domain. In this case, the difference in the action of DNA gyrase on the transcription of T7 and λ DNA would be a simple reflection of the fact that supercoiling activates some promoters but has no effect on others [11].

In any case, regardless of the particular mechanism, the stimulation of the transcription of linear DNA by DNA gyrase seems to be of interest and raises the question of the effect of DNA gyrase upon the replication and recombination of linear DNA.

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