Stimulation of RNA synthesis in isolated chromatin by an addition of DNA topoisomerases from cauliflower inflorescence

BY Hideki FUKATA and Hirosuke FUKASAWA

Biochemical Laboratory, Kobe Women's University, Suma-ku, Kobe 654

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ABSTRACT

The effects of DNA topoisomerases isolated from cauliflower inflorescence on *in vitro* RNA synthesis have been investigated using *Escherichia coli* RNA polymerase. When supercoiled pBR322 and pCaMV-S DNAs were used as the template, RNA synthesizing activity was fairly reduced by an addition of DNA topoisomerases. Whereas, a remarkable stimulation of RNA synthesis was observed when isolated cauliflower chromatin was used as the template by means of an addition of DNA topoisomerases.

DNA topoisomerases are enzymes able to interconvert various topological forms of DNA without altering the primary structure of DNA (Wang and Liu 1979) and thereby participate in a number of genetic processes. In prokaryotic cells, the importance of these enzymes in DNA replication (Gellert 1981; Kagni *et al.* 1984), in transcription (Akrigg and Cook 1980; Yang *et al.* 1979), and in recombination (Kikuchi and Nash 1979) has been suggested. However, the function of topoisomerases in eukaryotic cells is less well understood. The inflorescence of cauliflower exhibits striking hypertrophic differentiation with rapid growth by frequent mitotic divisions. Type-I and -II topoisomerases have been isolated from cauliflower head and characterized (Fukata and Fukasawa 1982, 1986). It is interesting to investigate the interrelationship between function of DNA topoisomerase and RNA synthesis in such hypertrophic tissues. In this report we describe that a remarkable *in vitro* stimulation of RNA synthesis observed in cauliflower chromatin in an experiment with *Escherichia coli* RNA polymerase added with DNA topoisomerases isolated from cauliflower inflorescence.

Cauliflower chromatin was isolated by the procedure described previously (Fukasawa and Hamada 1974) according to the method by Bonner *et al.* (1968) with some modifications. The soluble chromatin fraction in 10 mM Tris-HCl pH 8.0 with 0.25 M sucrose was centrifuged at $3,500\times g$ for 30 min, and the precipitate was used for each experiment. The DNA templates used in a preliminary experiments, done to find the effect of topoisomerases on RNA synthesis in supercoiled DNA, were pBR322 DNA and pCaMV-S DNA cloned by Hirochika *et al.* (1985). pCaMV-S DNA untwisted in various degrees were

prepared with topoisomerase from cauliflower in the presence of ethidium bromide. *E. coli* RNA polymerase used in the experiment was obtained from Pharmacia.

Decrease of RNA synthesizing activity of supercoiled DNAs by DNA topoisomerase

As a preliminary experiment, effects of cauliflower DNA topoisomerases on the RNA synthesis of supercoiled plasmid DNAs were investigated. As seen in Table 1, the template activities for RNA synthesis of DNAs pBR322 and pCaMV-S were fairly reduced by an addition of topoisomerase-I or -II; reduction stronger in pBR322 DNA than in pCaMV-S DNA, whereas calf-thymus DNA which seems to be linear form has shown no effect on RNA synthesis by an addition of topoisomerases. This results indicates that supercoiled DNAs are relaxed by topoisomerases and resulted in reduction of RNA synthesizing activity.

Table 1. *Effect of topoisomerase on transcriptional activity*

Topoisomerase	pBR322		pCaMV-S		calf-thymus	
	cpm	%	cpm	%	cpm	%
none	5000	100	7268	100	12016	100
Type-I	2878	58	5558	76	12075	100
Type-II	2866	57	5574	77	12769	106

Reaction mixture (25 μ l) contained: 50 mM Tris-HCl pH 7.5; 1 mM $MgCl_2$; 2 mM $MnCl_2$; 1 mM DTT; 150 mM KCl; 0.4 mM each of ATP, GTP, CTP and 0.2 mM 3H -UTP (0.5 μ Ci); 1 μ g of DNA; and 30 units of topoisomerase-I or -II. After incubation at 35°C for 10 min, 1 unit of RNA polymerase was added to the mixture. Incubation was continued for 10 min and 20 μ l each of the samples was put onto a paper disc, and the radioactivity was measured with a scintillation counter. One unit of topoisomerase activity is defined as the amount of enzyme required to relax 0.5 μ g of supercoiled pBR322 DNA fully in 10 min at 35°C.

Further to know the interrelationship between RNA synthesizing activity and DNA superhericity, the supercoiling of pCaMV-S DNA were made at various degrees by cauliflower DNA topoisomerase-II incubating in a standard reaction mixture for relaxation (Fukata and Fukasawa 1982) in the presence of various concentrations of ethidium bromide as indicated in the legend of Fig. 1. Template activities of thus obtained DNAs were examined. As seen in Fig. 1, DNA higher the degree of supercoiling gave the higher template activity. This result agrees with the fact that supercoiled DNA is required for transcription of most genes in prokaryotic cells (Smith *et al.* 1978; Yang *et al.* 1979).

Fig. 1. Interrelationship between RNA synthesis (transcription) and superhelicity of pCaMV-S DNA. Various supercoiled DNAs were prepared from the incubation of cauliflower DNA topoisomerase-II with pCaMV-S DNA in the reaction mixture for relaxation, containing 0.2-1.0 $\mu\text{g/ml}$ of ethidium bromide at intervals of 0.2 $\mu\text{g/ml}$ (lane, a-e), and purified by extraction with *n*-butanol and precipitation with ethanol. RNA synthetic activity was measured with the incorporation of ^3H -UMP using one unit of *E. coli* RNA polymerase at 35°C for 10 min, in the same reaction medium as described in Table 1. Lane a-e, partially relaxed DNA; lane f, native supercoiled DNA.

Stimulation of chromatin template activity for RNA synthesis by DNA topoisomerase

Template activity for RNA synthesis of isolated cauliflower chromatin was investigated in the same reaction medium as used for the experiment of supercoiled DNAs described above. As seen in Fig. 2, it is surprising that remarkable increases of RNA synthetic activity were observed in accordance with the quantity of DNA topoisomerase added to the reaction medium. Since the unit value of topoisomerase-I solution used in this experiment was 5-times higher than that of topoisomerase-II, topoisomerase-II would be more active to stimulating RNA synthesis than topoisomerase-I in chromatin. In the experiment simultaneously done on the chromatin fraction precipitated at $70,000\times g$, the stimulative effect was not so remarkable (about 20% stimulation, data not shown). This means that the stimulative effect of RNA synthesis by topoisomerase is largely dependent on the conditions of chromatin used.

Purified cauliflower topoisomerases catalyze several types of reaction, such as relaxation, catenation and decatenation, and unknotting of DNA. Moreover, cauliflower topoisomerases can introduce supercoils into relaxed DNA with histone *in vitro* (unpublished data). However, it is not known how topoisomerases act on chromatin, and stimulative mechanism of RNA synthesis on isolated chromatin by topoisomerases is not yet clear at present time.

Fig. 2. Stimulation of RNA synthesis of isolated chromatin by an addition of DNA topoisomerase-I or -II from cauliflower inflorescence. Reaction mixture was the same as described in Table 1, excepting that supercoiled DNA was replaced with chromatin (included 1 μ g of DNA). In topoisomerase-I and -II, 1 μ l corresponds with 10 and 2 units, respectively. ●—●: chromatin + topoisomerase-I; ○---○: chromatin + topoisomerase-II; ●---●: calf-thymus DNA + topoisomerase-I or -II.

Luchnik *et al.* (1982) described in the study on SV 40 minichromosomes that histones are dissociated from the relaxed DNA after treatment with topoisomerase of Ehrlich ascites carcinoma cells. Therefore, it is considered in the present experiment that removal or loosening of histone from the presumably condensed nucleosomes could be done by an addition of topoisomerases with unusually high concentration which induces the exposure of supercoiled DNA in the domains. Further precise experiments with various state of chromatins are in progress to disclose the stimulative mechanism.

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