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Molecular Biology

Endonuclease Like Activity of DNA Topoisomerase II Associated with Nuclear Matrix at Low pH Value

Tengiz Zaalishvili[*], Ketevan Kutalia[**], Ketevan Kolkhidashvili[**], Dina Margiani[**], Tinatin Eristavi[**]

[*] *Academy Member, I.Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*
[**] *I.Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*

It has been shown that DNA topoisomerase activitiy of rat liver nuclear matrix isolated by high salt extraction method can only split superhelical plasmid DNA at low acidic pH producing single strand cleavages like endonuclease without religation possibility of DNA. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Liver, nuclei, nuclear matrix, DNA topoisomerase

The non-chromatin protein skeleton of the nucleus – nuclear matrix, to which periodic and specific attachments of chromatin fibers create the chromatin loop domains, is probably implicated in nuclear processes. The obtained data show, that processes, such as DNA replication, transcription, RNA processing and transport, DNA repair and regulation of DNA superhelicity are associated with nuclear matrix [1-4].

On the other hand, the topological structure of DNA in the cell is modulated by ubiquitous enzymes known as DNA topoisomerases. In general DNA topoisomerases are divided in two classes, type I and type II, depending on whether they cleave one or two strands of DNA, respectively. DNA topoisomerase I relaxes supercoiled template by nicking a single strand of DNA double strand allowing one end to rotate with respect to the other around the intact strand or by passing one strand through the break. DNA topoisomerase II cleaves both strands of DNA duplex and passes a second intact duplex to the transient break [5,6]. Topoisomerases solve the topological problems associated with DNA replication, transcription, recombination and chromatin remodeling [5-8].

It is well known that acidic pH plays an important role in cell death during various pathological states (including ischemia and cancer) and on the other hand, involved in carcinogenesis [9,10]. Based on above mentioned the aim of this investigation was to determine how nuclear matrix associated DNA topoisomerase worked at acidic condition.

Materials and Methods

White rats weighing ~ 120-130 g were used. Liver nuclei were isolated by a simple two-step method of Georgiev et al. [11] with some modifications. Minced tissue was homogenized in twofold volume

solution containing 10 mM Tris –HCl (pH 7.2), 2.2 M Sucrose, 4 mM $MgCl_2$ in Potter-Elvehjem homogenizer with teflon pestle rotating at a speed of 700-800 rpm. The homogenate was filtered through 4 layers of gauze and centrifuged at 6 000 g for 40 min. The precipitate obtained from 10 g of tissue was suspended in 6-8 ml of buffer solution containing ~ 10 mM Tris-HCl (pH 7.4), 0.32 M Sucrose, 3 mM $MgCl_2$ and layered on 16 ml of the same solution containing 1.1 M Sucrose and centrifuged at 6 000 g for 10 min.

Nuclear matrices were prepared according to Berezney and Coffey [12] and Berezney [13] with some changes. Pure liver nuclei (2 mg protein/ml) were endogenously treated in a buffer solution containing 20 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.25 M Sucrose at 30°C for 30 min and after centrifugation of suspension at 3000 g for 15 min. the nuclei were suspended in 10 mM Tris-HCl (pH 7.4), 0.2 mM $MgCl_2$ (2 mg protein/ml) and centrifuged at 3000 g for 15 min. After centrifugation the nuclear material were extracted by 10 mM Tris-HCl (pH 7.4), 2 M NaCl), 0.2 mM $MgCl_2$ (2 mg protein/ml 15 min. and centrifuged at 10 000 g for 15 min. The procedure was repeated twice. The pellet was extracted with 10 mM Tris-HCl (pH 7.4), 0.2 mM $MgCl_2$ and 0.1% Triton X-100 (4 mg protein/ml) and two more times with the same buffer solution (2 mg protein/ml) which did not contain detergent. Centrifugation during washing was conducted at 5 000 g for 15 min.

The solutions during preparation of nuclei and nuclear matrices contained a protease inhibitor – phenylmethylsulfonyl fluoride in a concentration of 0.2 mM. All procedures except the cases indicated in text were carried out at 1-3°C.

For the detection of DNA topoisomerase activity of the nuclear matrix a reaction mixture with a volume 20 μl containing 25 mM Tris-HCl (pH 8.0) or 25 mM MES (pH 5.5) buffer 0.15 M NaCl, 5 mM $MgCl_2$, and 0.5 μg pUC 19 DNA in the presence or absence of 1 mM ATP was used. The reaction area contained 2-4 μg protein of nuclear matrix. After the incubation of samples at 30°C for 20 min. they were treated with 1% sarcosyl and 100 μg/ml protease K at 50°C for 60 min. After addition to this mixture 1/4 volume of 5 x Green GoTaq Reaction Buffer the samples were applied to horizontal 1% agarose gel and electrophoresis was conducted at 65 mA in a buffer solution containing 40 mM Tris-acetate and 1 mM EDTA (pH 8.2) at the room temperature for 2.5-3 h.

The gels were stained with intercalator – ethidium bromide (0.5 μg/ml) for 15 min. and visualized by illumination with ultraviolet lamp [14].

Two-dimensional agarose gel electrophoresis of DNA in the second dimension was carried out perpendicularly to the first dimension for 50-60 min. in the same conditions after staining this gel with ethidium bromide.

Plasmid DNA was isolated from transformed E.coli cells containing the plasmid pUC 19 [15].

The protein concentration was determined according to Bradford [16].

Results and Discussion

It is established that purified DNA topoisomerase II can only split one strand of double stranded DNA without religation at acidic pH [9, 17]. On the other hand, it is not known how DNA topoisomerase II associated with nuclear matrix acts at low pH value. Based on the above mentioned we incubated plasmid DNA with the isolated matrix in reaction medium at different pH values to determine how nuclear matrix DNA topoisomerase works at low acidic pH.

One-dimensional agarose gel electrophoresis showed that nuclear matrix associated DNA topoisomerase converted plasmid DNA at pH -5.5 identically as in the case of higher pH – 8.0 value (Fig. 1).

Analysis of topoisomerase reaction product by two-dimensional agarose gel electrophoresis (Fig. 2) showed that at acidic pH value in difference to higher pH – 8.0 value nicked – open circular DNA

is produced instead of relaxed-closed circular form of DNA. So, at acid pH topoisomerase functions like endonuclease, it cannot religate DNA strand break as in the case of higher pH value.

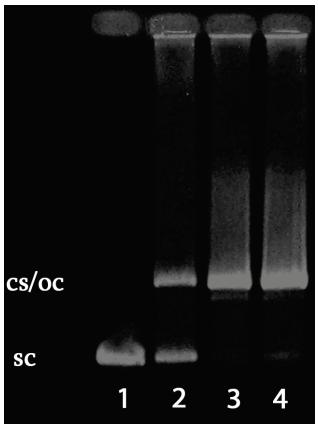

Fig. 1. The nuclear matrix DNA topoisomerase activities at different pH values analyzed by one-dimensional agarose gel electrophoresis of plasmid DNA at different pH values.
1. Control – plasmid pUC19 DNA;
2. DNA topoisomerase activity in 25mM Tris-HCl (pH 8.0) buffer;
3. DNA topoisomerase activity in 25mM MES (pH 5.5) buffer;
4. DNA topoisomerase activity in 25mM MES (pH 5.5) buffer containing 1 mM ATP.

Based on the data that isolated rat liver nuclear matrix contains DNA topoisomerase I as well as DNA topoisomerase II [7] and that purified DNA topoisomerase I at acidic pH in difference with DNA topoisomerase II can split and religate DNA [18] we can conclude that nuclear matrix associated DNA topoisomerase II is a reason of producing single strand DNA cleavages of plasmid DNA.

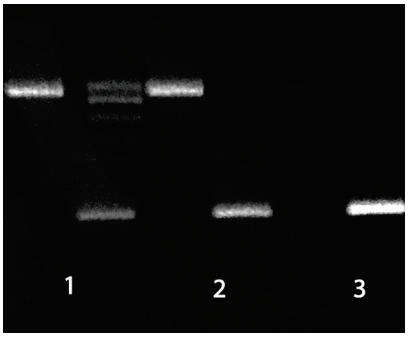

Fig. 2. The determination of DNA topoisomerase reaction products at different pH values by two-dimensional gel electrophoresis of plasmid puC19 DNA.
1. DNA topoisomerase activity in 25 mM Tris-HCl (pH 8.0) buffer;
2. DNA topoisomerase activity in 25 mM MES (pH 5.5) buffer containing 1 mM ATP;
3. Control – plasmid pUC19 DNA.

The obtained data indicate that nuclear matrix DNA topoisomerase II changes its structure at low pH that causes the changing of double strand DNA cleavage into single strand DNA cleavage and elimination its ATP dependent religation activity.

As it was mentioned above acidic pH is associated with cancer. On the other hand DNA topoisomerase II plays an important role in producing DNA cleavages at low acidic pH which may induce mutation and cytotoxity and develop cancer. It is suggested that at acidic area chromosomal breaks occur in matrix or scaffold attachment region (MAR/SAR) [10].

The present work demonstrates that nuclear matrix DNA topoisomerase II may play significant role in various pathological processes.

მოლეკულური ბიოლოგია

ბირთვულ მატრიქსთან ასოცირებული დნმ ტოპოიზომერაზა II-ის ენდონუკლეაზის მსგავსი აქტივობა დაბალ pH-ზე

თ. ზაალიშვილი*, ქ. კუტალია**, ქ. კოლხიდაშვილი**, დ. მარგიანი**, თ. ერისთავი**

* *აკადემიის წევრი, ი. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო*
** *ი. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო*

ნაჩვენებია, რომ მაღალ იონურ ძალაზე იზოლირებულ ვირთაგვას ღვიძლის მატრიქსთან ასოცირებულ დნმ ტოპოიზომერაზას დაბალ მჟავე pH-ზე შეუძლია სუპერსპირალური პლაზმიდური დნმ-ის მხოლოდ გახლეჩა ერთძაფიანი ნახლეჩების წარმოქმნით, მსგავსად ენდონუკლეაზისა, დნმ-ის მოლეკულის რელიგაციის უნარის გარეშე.

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