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DNA Topoisomerase Activities of Nuclear Matrices Isolated by Different Slow (Two Days) and Fast (One Day) Methods from Rat Liver

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ABSTRACT. DNA topoisomerase activities of nuclear matrices isolated from rat liver by slow (two days) and fast (one day) methods were determined. It was established that nuclear matrix isolated from rat liver by slow (two days) method revealed only DNA topoisomerase I activity, while nuclear matrix isolated by fast (one day) method exhibited both DNA topoisomerase I as well as DNA topoisomerase II activities. It is supposed that during isolation of nuclear matrix using slow (two days) method protease degradation of DNA topoisomerase II takes place. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: liver, nuclei, nuclear matrix, DNA topoisomerase

In the cell the topological structure of DNA 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 [1, 2]. Topoisomerases solve the topological problems associated with DNA replication, transcription, recombination and chromatin remodeling [1-4]. On the other hand, a 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 the nuclear matrix [5-8].

Based on above mentioned the aim of this investigation was to study the association of DNA topoisomerase I and II activities with nuclear matrices isolated from rat liver using fast (one day) and slow (two days) methods.

Materials and Methods

White rats weighing ~ 120-140 g were used. Liver nuclei were isolated by a simple two-step method of Georgiev et al.[9] 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 containing10 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 [10] and Berezney [11] with some changes. Liver nuclei (2 mg protein/ml) were endogenously digested by incubation at 22^0 C for 15 min in a buffer solution containing 50 mM Tris-HCl (pH 7.4), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 150 mM Sucrose. Then, the nuclear suspension was centrifuged at 3 000 g for 10 min and the pellet was suspended in a buffer solution containing 10 mM Tris-HCl (pH 7.4), 0.2 mM $MgCl_2$(2 mg protein/ml) and incubated at 2^0 C for 16 h. After centrifugation of suspension at 3 000 g for 15 min the nuclear material were extracted with 10 mM Tris-HCl (pH 7.4), 2 M NaCl (2 mg protein/ml) for 15 min and centrifuged at10 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.

While isolation of nuclear matrix by fast (one day) method 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 30^0 C for 30 min and after centrifugation of suspension at 3 000 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) for 15 min and centrifuged at 3 000 g for 15 min. After this the nuclear material was extracted as described above.

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 1-3^0 C.

For the detection of DNA topoisomerase I activity of the nuclear matrix a reaction mixture with a volume 20 μl containing 25 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 5 mM $MgCl_2$ or 1 mM EDTA (pH 8.0) and 0.5 μg pUC 19 DNA were used. To reveal nuclear matrix DNA topoisomerase II activity the same reaction mixture containing 5 mM $MgCl_2$ and 1 mM ATP was used. The reaction area contained 2 μg and 4 μg protein of nuclear matrix isolated by fast and slow methods correspondingly.

After the incubation of samples at 30^0 C for 20 min they were treated with 1% sarkosyl and 100 μg/ml protease K at 50 ^{0} C for 60 min. After addition to this mixture 1 /4 volume of 5xGreen 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 [12].

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 the gel with ethidium bromide.

Plasmid DNA was isolated from transformed *E.coli* cells containing the plasmid pUC19 [13].

The protein concentration was determined according to Bradford [14].

Results and Discussion

It is well known that type I DNA topoisomerase of eukaryotic cells without ATP makes a single strand break on supercoiled DNA, while type II DNA topoisomerase makes a double strand break only in the presence of Mg^{2+} and ATP [15, 16]. So, DNA topoisomerase I is an ATP-independent enzyme, and it does not require divalent cation (Mg^{2+}) for activity, although Mg^{2+} stimulates topoisomerase I activity [16]. These enzymatic properties allow for a clear distinction between topoisomerase I and topoisomerase II which is active exclusively in the presence of Mg^{2+} and ATP.

Based on the above mentioned to distinguish type I and type II DNA topoisomerases of liver nuclear matrix isolated by slow (two days) and fast (one day) methods topoisomerase activity was determined by relaxation of supercoiled pUC 19 DNA in the presence of EDTA (1mM) without Mg^{2+} and ATP; in the presence of Mg^{2+} without ATP and in the presence of Mg^{2+} and ATP.

Fig.1 depicts DNA topoisomerase activity of nuclear matrix isolated from rat liver by slow (two days) method. It can be seen that nuclear matrix reveals topoisomerase activity without cations in the presence of EDTA. This fact indicates that the nuclear matrix contains DNA topoisomerase I. Fig.1 also shows that divalent cation (Mg^{2+}) in the presence of ATP stimulates nuclear matrix DNA topoisomerase activity.

On the other hand, the level of DNA topoisomerase activity does not change by exclusion ATP from the reaction medium containing Mg^{2+}. This indicates that nuclear matrix isolated by slow (two days) method does not contain DNA topoisomerase II and shows that divalent cation stimulates DNA topoisomerase I. The absence of DNA topoisomerase II in isolated rat liver nuclear matrix is also confirmed by the influence of chemotherapeutic agent - topotecan on the matrix topoisomerase activity. The DNA topoisomerase drugs in current clinical use influence these enzymes in a very selective manner. These agents including the eukaryotic DNA topoisomerase I drugs, such as camptothecin and its analogs - irinotecan and topotecan and the DNA topoisomerase II drugs - doxorubicin, etoposide, teniposide,etc - convert their target topoisomerases to DNA- damaging agents. Normally, both types of enzymes bind to and cleave DNA by forming intermediate covalent enzyme - DNA complex and these scissions are religated after relaxation of DNA. However, chemotherapeutic agents (topoisomerase poisons) stabilize covalent enzyme - DNA complex by forming drug - enzyme - DNA complex and prevent the subsequent DNA - resealing step catalyzed by DNA topoisomerases [16].

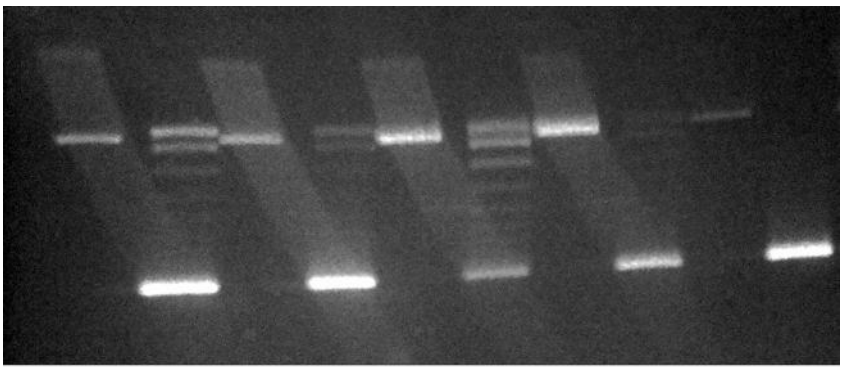
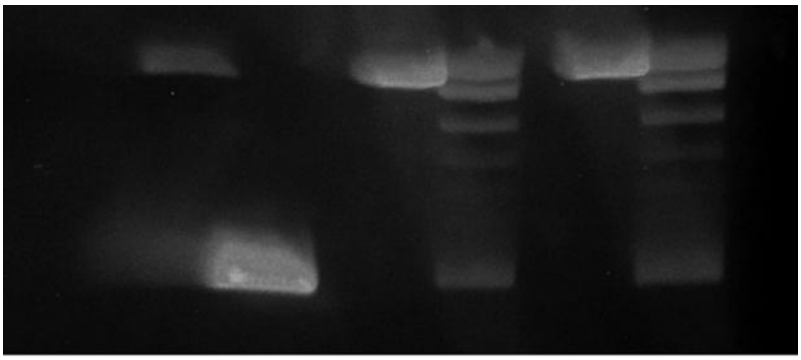
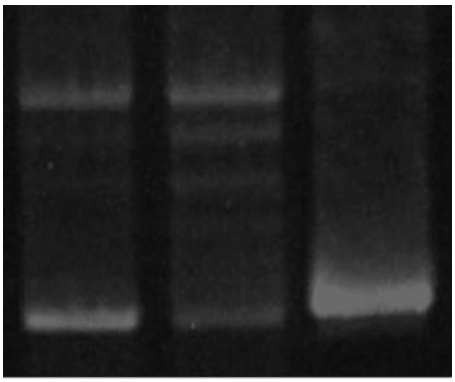


Fig. 1. The determination of DNA topoisomerase activity of nuclear matrix isolated from rat liver by slow (two days) method using two - dimensional agarose gel electrophoresis of plasmid pUC19 DNA.
DNA topoisomerase activity in the presence of 1 mM EDTA (1), 1 mM EDTA and 50 μM topotecan (2), 5 mM $MgCl_2$ and 1 mM ATP (3), 5 mM $MgCl_2$, 1 mM ATP and 50 μM topotecan (4), control - plasmid pUC19 DNA (5).



1 2 3

Fig. 2. Influence of etoposide - VP16 on DNA topoisomerase activity of nuclear matrix isolated from rat liver by slow (two days) method using two - dimensional agarose gel electrophoresis of plasmid pUC19 DNA
DNA topoisomerase activity in the presence of control - plasmid pUC19 DNA (1), 5 mM $MgCl_2$ and 1 mM ATP (2), 5 mM $MgCl_2$,1 mM ATP and 50 μM etoposide -VP16 (3). The reaction mixtures contained 2.5% dimethyl sulfoxide.



1 2 3

Fig. 3. The determination of DNA topoisomerase activity of nuclear matrix isolated from rat liver by fast (one day) method using one - dimensional agarose gel electrophoresis of plasmid pUC19 DNA.
DNA topoisomerase activity in the presence of 5 mM $MgCl_2$ (1), 5 mM $MgCl_2$ and 1 mM ATP (2), control - plasmid pUC19 DNA (3).

Fig.1 presents the influence of chemotherapeutic agent-topotecan on isolated by slow (two days) method nuclear matrix DNA topoisomerase activity. As one can see the part of pUC19 DNA molecules are relaxed and religated (faster migrated) by topoisomerase action and the part of DNA molecules are only relaxed (slowly migrated). It can also be seen that topotecan completely inhibits religation step of nuclear matrix topoisomerase reaction which was determined in the presence of Mg^{2+} and ATP showing that isolated nuclear matrix contained only DNA topoisomerase I. Fig.2 shows that specific inhibitor of DNA topoisomerase II chemotherapeutic agent etoposide - VP16 has no effect on religation step of topoisomerase activity, wich indicated also about the absence of DNA topoisomerase II in nuclear matrix isolated by slow method.

As regards to the DNA topoisomerase activity of nuclear matrix isolated by fast (one day) method it must be mentioned, that this matrix contains both DNA topoisomerases: DNA topoisomerase I and DNA topoisomerase II. As one can see from Fig.3 nuclear matrix isolated by fast method exhibited DNA topoisomerase activity in the presence of Mg^{2+} and this activity is stimulated by ATP as it was mentioned earlier is a characteristic property of DNA topoisomerase II.

So, we can conclude that the nuclear matrix isolated by slow (two days) method contains only DNA topoisomerase I, while nuclear matrix isolated by fast (one day) method contains both:DNA topoisomerase I and DNA topoisomerase II. The reason for this may be the protease degradation of DNA topoisomerase II during the isolation of nuclear matrix by slow method. This assumption is supported by protein recovery of nuclear matrices. Nuclear matrix obtained by slow method contained ~3% of nuclear protein while nuclear matrix isolated by fast method contained ~10% of nuclear protein.

მოლეკულური ბიოლოგია

ვირთაგვას ღვიძლიდან სხვადასხვა ნელი (ორდღიანი) და სწრაფი (ერთდღიანი) მეთოდებით გამოყოფილი ბირთვული მატრიქსების დნმ ტოპოიზომერაზული აქტივობები.

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განსაზღვრულია სხვადასხვა ნელი (ორდღიანი) და სწრაფი (ერთდღიანი) მეთოდებით გამოყოფილი ვირთაგვას ღვიძლის ბირთვული მატრიქსების დნმ ტოპოიზომერაზული აქტივობები. დადგენილია, რომ ნელი(ორდღიანი) მეთოდით გამოყოფილი ბირთვული მატრიქსი ავლენს მხოლოდ დნმ ტოპოიზომერაზა I-ის აქტივობას, მაშინ როდესაც სწრაფი (ერთდღიანი) მეთოდით გამოყოფილი ბირთვული მატრიქსი ამჟღავნებს როგორც დნმ ტოპოიზომერაზა I-ის, ასევე დნმ ტოპოიზომერაზა II-ის აქტივობებს. ნავარაუდევია, რომ ბირთვული მატრიქსის ნელი (ორდღიანი) მეთოდით გამოყოფის პროცესში ადგილი აქვს დნმ ტოპოიზომერაზა II-ის პროტეაზულ დეგრადაციას.

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