საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 19(193), №3, 2025
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 19(193), no. 3, 2025

Molecular Biology

Influence of Resveratrol on Rat Liver Nuclear Matrix DNA Topoisomerase II Activity

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Abstract. Resveratrol is a polyphenol that occurs in various plants and possesses cardioprotective, neuroprotective, anticarcinogen and other beneficial properties. On the other hand, purified DNA topoisomerase II which modulates topological state of DNA and participates in various genetic processes has been added to the list of potential resveratrol targets as a possible explanation for the anticancer effects of the resveratrol. The nonchromatin protein skeleton of the nuclear matrix to which periodic and specific attachments of chromatin fibers create loop domains is probably implicated in various processes, such as DNA replication, transcription, RNA processing and transport, DNA repair and the regulation of DNA superhelicity are associated with nuclear matrix – skeleton. Based on the above mentioned, the aim of this work was to determine how nuclear matrix – skeleton associated with DNA topoisomerase II works in the presence of resveratrol. In our experiments, nuclear matrices were isolated from white rat liver tissue. The samples were digested by DNA-ase 1 and subsequent high salt extraction after preliminary treatment of the nuclei with DNA-ase 1. The protein concentration was determined according to Bradford. For determination of DNA topoisomerase activity of nuclear matrices skeleton containing samples were applied to horizontal agarose gel and electrophoresis was conducted, stained with ethidium bromide gels and visualized under ultraviolet illumination. Images were analyzed using ImageJ software. The present work revealed that resveratrol affects not only purified DNA topoisomerase II but significantly inhibits DNA topoisomerase II activity of the nuclear matrix isolated from rat liver *in vitro*. These results suggest that nuclear matrix DNA topoisomerase II ATP domain dimerization interface is not masked (as in the case of purified DNA topoisomerase II) by other components of the nuclear matrix and is still acceptable for interaction with resveratrol. *© 2025 Bull. Georg. Natl. Acad. Sci.*

Keywords: liver, nuclei, nuclear matrix, DNA topoisomerase, resveratrol

Introduction

Resveratrol is a polyphenol that among other plant sources occurs in grapes and by this reason red wines also contain it. Resveratrol is thought to be a reason of the “French Paradox” which associates red wine consumption to the low incidence of cardiovascular diseases. The interest towards resveratrol has increased not only by its cardioprotective properties, but also due to its neuroprotective, anti-inflammatory, antioxidant, anti-carcinogenic, anti-aging and other actions (Neves et al., 2012, Novelle

et al., 2015). 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 various 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 (Nickerson et al., 2001; Linnemann et al., 2009; Arranda et al., 2014, Martinovic et al., 2015; Razin et al., 2022). On the other hand, the topological structure of DNA in the cell is modulated by ubiquitous enzymes known as DNA topoisomerases. DNA topoisomerases are divided in various classes. Type I and type II DNA topoisomerases cleave one or two strands of DNA, respectively. It must be mentioned that DNA topoisomerase II which modulates topological state of DNA has been added to the list of potential resveratrol targets as a possible explanation for the anticancer effects of the resveratrol (Jo et al., 2006; Leone et al., 2010; Lee et al., 2017). Based on the above mentioned the aim of this work was to determine how nuclear matrix – skeleton associated DNA topoisomerase II works in the presence of resveratrol.

Materials and Methods

Isolation of rat liver nuclei. White rats weighing ~ 120-140 g were used. Liver nuclei were isolated by a simple two-step method of Georgiev et al. (1960) with some modifications. Minced liver tissue was homogenized in two-fold 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.

Preparation of nuclear matrices. Nuclear matrices were prepared according to Berezney and Coffey (1977) and Berezney (1980) with some changes. Pure liver nuclei (2 mg protein/ml) were endogenously treated in a buffer solution containing 20 mM Tris-HC1 (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 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) and centrifuged at 3 000 g for 15 min. After centrifugation the nuclear material were extracted by 10 mM Tris-HCl (pH 7.4), 0.2 mM $MgCl_2$, 2 M NaCl (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.

All solutions during preparation of nuclei and nuclear matrices contained a protease inhibitor – phenylmethylsulphonyl fluoride (PMSF) in a concentration of 0.2 mM. All procedures except the cases indicated in text were carried out at 2-4°C.

The isolated nuclear matrix contained ~ 10% of nuclear protein.

Plasmid DNA purification. Plasmid pUC19 containing E.coli DH5alfa was grown overnight (O/N) in 3 ml of LB-medium supplemented with ampicillin (100 μkg/ml). Plasmid DNA was isolated by Fast-n-Easy Plasmid Mini-Prep Kit (Jena Biosceince) according to manufacturer's instructions. Briefly, 1 ml of bacterial culture was harvested by centrifugation at 5 000 g for 3 min at room temperature (RT) and the pellet was resuspended in 300 μl of Lysis Buffer. After vortexing 300 μl of

RNase containing Neutralization Buffer the sample was centrifuged at 10 000 g for 5 min at RT. The supernatant was applied into the activated Binding column and centrifuged at 10 000 g for 30 sec. After washing the column with 500 µl of Washing Buffer plasmid DNA was eluted with 30 µl sterile ddH_2O. The purified plasmid DNA concentration was determined using

NanoDrop 1 000 spectrophotometer. Plasmid DNA was also isolated from small-scale (2 ml) bacterial cultures by alkaline lysis with sodium dodecyl sulfate Green et al., 2016).

Determination of DNA topoisomerase activity of nuclear matrices. For the determination of DNA topoisomerase 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 $MgCl_2$, 0.5 µg pUC19 DNA in the presence or absence of 1 mM ATP was used. The reaction mixture contained 3 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 stop-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 photographed under ultraviolet illumination (Nishizava et al., 1984). Images were analyzed by ImageJ software.

The estimation of quantities of protein. The protein concentration was determined according to Bradford (1976).

Results and Discussion

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 various 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 (Nickerson et al., 2001; Linnemann et al., 2009; Arranda et al., 2014; Martinovic et al., 2015; Razin et al., 2022). On the other hand, the topological structure of DNA in the cell is modulated by ubiquitous enzymes known as DNA topoisomerases. DNA topoisomerases are divided in various classes. Type I and type II DNA topoisomerases 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 (Schoeffler et al., 2008; Baranell et al., 2016). Topoisomerases solve the topological problems associated with DNA replication, transcription, recombination and chromatin remodeling (Schoeffler et al., 2008; Baranell et al., 2016; Zaalishvili et al., 2017; Pommier et al., 2016).

It is well known that DNA topoisomerase I of eukaryotic cells without ATP makes a single-strand break on supercoiled DNA, while DNA topoisomerase II makes a double strand break only in the presence of Mg^{2+} and ATP. So, DNA topoisomerase I is an ATP-independent enzyme that does require a divalent cation (Mg^{2+}) for activity, although Mg^{2+} stimulates DNA topoisomerase I (McKie et al., 2021; Nitiss et al., 2012).

Recently, it was shown that resveratrol modulates – inhibits purified DNA topoisomerase II (Lee et al., 2017), but it is not known how nuclear matrix associated DNA topoisomerases I and II works in the presence of resveratrol.

To determine nuclear matrix DNA topoisomerase I and II activities in the presence of resveratrol, we incubated nuclear matrices with various concen-

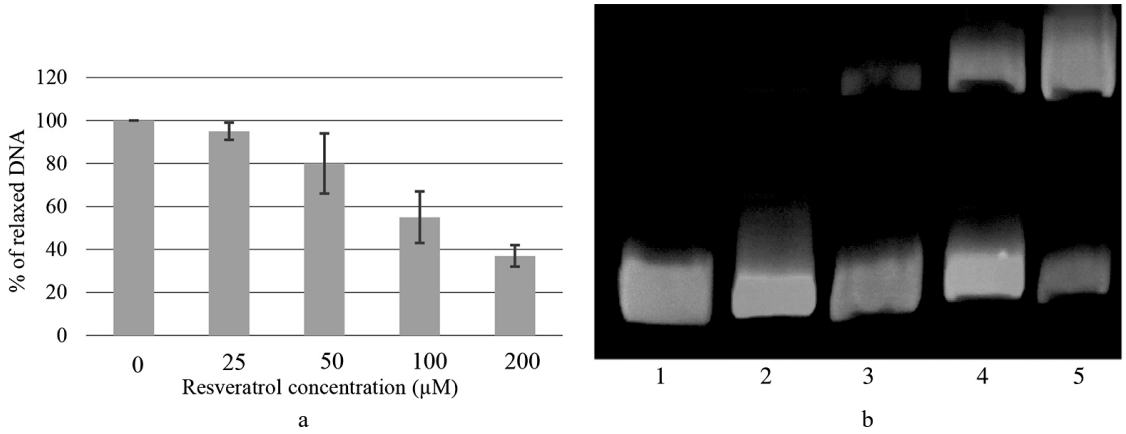

Figure. Influence of resveratrol on rat liver nuclear matrix DNA topoisomerase II activity. (a) The influence of various concentrations of resveratrol on DNA topoisomerase II relaxation activity using densitometry data of relaxed DNA after agarose gel electrophoresis of plasmid pUC19 DNA. (b) Representative image showing the influence of various concentrations of resveratrol on DNA topoisomerase II relaxation activity of isolated rat liver nuclear matrix. 1 – Supercoiled pUC19 DNA, 5 – control sample; 2-4 – DNA topoisomerase activities after incubation of matrixes with 200 μM, 100 μM and 50μM resveratrol respectively.

trations of resveratrol in the presence or absence of ATP. Our results show that resveratrol inhibits nuclear matrix DNA topoisomerase activity when topoisomerase activity assay was carried out in the presence of ATP. As Fig. 1 shows, resveratrol inhibits nuclear matrix DNA topoisomerase II activity starting from 50 μM and reaches approximately 63% when resveratrol concentration reaches 200 μM. Alternatively, no changes in topoisomerase activity were observed when ATP was omitted from reaction buffer (data not shown). So, we can conclude that resveratrol inhibits nuclear matrix DNA topoisomerase II, but not topoisomerase I activity.

Conclusion

Many polyphenols (including flavonoids) possess dihydroxyphenyl groups that resemble the six – membered ring of resveratrol. Scientists speculate that this moiety may be the reason that polyphenol agents act as DNA topoisomerase II antagonists (Lee et al., 2017). In this case, natural products like (including) resveratrol could allosterically influence DNA topoisomerase II function through the highly-conserved binding site at the ATPase domain dimerization interface. Based on our results, we can assume that nuclear matrix topoisomerase II ATPase domain dimerization interface is not masked by other components and remains acceptable for interaction with resveratrol.

მოლეკულური ბიოლოგია

რეზვერატროლის ზეგავლენა ვირთაგვას ღვიძლის ბირთვული მატრიქსის დნმ ტოპოიზომერაზა II-ის აქტივობაზე

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** აკადემიის წევრი, სსიპ ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, გენომის სტრუქტურის და ფუნქციის ლაბორატორია, თბილისი, საქართველო*
*** სსიპ ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, გენომის სტრუქტურის და ფუნქციის ლაბორატორია, თბილისი, საქართველო*

რეზვერატროლი წარმოადგენს პოლიფენოლს, რომელიც გვხვდება სხვადასხვა მცენარეში და გააჩნია კარდიოპროტექტორული, ნეიროპროტექტორული, ანტიკანცეროგენული და სხვა თვისებები. მეორე მხრივ, გასუფთავებული დნმ ტოპოიზომერაზა II (რომელიც მოდულირებს დნმ-ს ტოპოლოგიურ მდგომარეობას და მონაწილეობს სხვადასხვა გენეტიკურ პროცესში) დაემატა რეზვერატროლის პოტენციურ სამიზნეთა რიცხვს, რეზვერატროლის ანტისიმსივნური მოქმედებების ახსნას. ზემოაღნიშნულიდან გამომდინარე, მოცემული შრომის მიზანი იყო დაგვედგინა, როგორ მუშაობს ბირთვულ მატრიქსთან – ჩონჩხთან ასოცირებული დნმ ტოპოიზომერაზა II რეზვერატროლის თანაობისას. ბირთვული მატრიქსის არაქრომატინული ცილოვანი ჩონჩხი, რომელთანაც ქრომატინის ფიბრილების სპეციფიკური დაკავშირებები წარმოქმნის ყულფურ დომენებს, სავარაუდოდ, ჩართულნი არიან სხვადასხვა ბირთვულ პროცესებში. მიღებული მონაცემები აჩვენებს, რომ ისეთი პროცესები, როგორიცაა დნმ-ის რეპლიკაცია, ტრანსკრიფცია, რნმ-ის პროცესინგი და ტრანსპორტი, დნმ-ის რეპარაცია და დნმ-ის სუპერსპირალობის რეგულაცია ასოცირებულია ბირთვულ მატრიქსთან – ჩონჩხთან. ექსპერიმენტებში ვიყენებდით ბირთვულ მატრიქსებს, რომლებიც იზოლირებული იყო თეთრი ვირთაგვების ღვიძლიდან გამოყოფილი და გასუფთავებული ბირთვების დნმ-აზა 1-ით, წინასწარი დამუშავებისა და მაღალი კონცენტრაციის მარილით შემდგომი ექსტრაჰირებით. ბირთვულ მატრიქსთან ასოცირებული დნმ ტოპოიზომერაზული აქტივობის განსაზღვრისათვის სარეაქციო სინჯები დაიტანებოდა აგაროზის ჰორიზონტალურ გელზე, იღებებოდა ეთიდიუმ ბრომიდით, თავსდებოდა ულტრაიისფერი გამოსხივების ქვეშ და ფოტოგრაფირდებოდა. გელის მიღებული სურათები ანალიზდებოდა ImageJ software პროგრამის გამოყენებით. მიღებულმა მონაცემებმა გამოავლინა, რომ რეზვერატროლი გავლენას ახდენს არამარტო გასუფთავებულ დნმ ტოპოიზომერაზა II-ზე, არამედ მნიშვნელოვნად აინჰიბირებს ვირთაგვას ღვიძლიდან იზოლირებულ ბირთვულ მატრიქსთან ასოცირებულ დნმ ტოპოიზომერაზა-2-ს. შედეგად, შეგვიძლია დავასკვნათ, რომ ბირთვული მატრიქსის დნმ ტოპოიზომერაზა II-ის ATP დომენის დიმერიზაციის ზედაპირი თავისუფალია (როგორც გასუფთავებული დნმ ტოპოიზომერაზა II-ის შემთხვევაში), არ არის დაკავებული მატრიქსის რომელიმე სხვა კომპონენტით და, შესაბამისად, მისაწვდომია რეზვერატროლისთვის.

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Received March, 2025