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Z u r a b T s e t s k h l a d z e

Study of properties and biological role of nuclear matrix poly(ADP-ribose)polymerase in eukaryotic cells

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The Abstract has been dispatched ---------- 2006

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General Description of the Work

Urgency of the subject. One of the central problem of modern biology is to understanding the structural and functional organization of long DNA molecules in eukaryotic cell nuclei.

DNA is represented in the nucleus in complex with other proteins forming super helical fibrils of 30 nm diameter, which are periodically folded in loops and with the ends are attached to the nuclear matrix. Topologically independent loops vary in size from 5 to 200 kb. Conversion of topological DNA isomers is performed through double-strand breaks by DNA-topoisomerases associated with sites, where loops are attached to the matrix. Since topological transformations play very important role in biological processes, DNA-topoisomerases are in the focus of interest of investigators **(Razin S., et al., 2001).**

Posttranslation modifications of nuclear proteins in eukaryotic cells is also tightly connected with genetical processes. The enzyme poly(ADP-ribose)polymerase (PARP), converting NAD molecules into $(ADP\text{-}ribose)_n$ and covalently or noncovalently transferring them on different proteins, is the enzyme accomplishing one of such posttranslational modifications of proteins. High negative charge of a polymer significantly affects function of the target proteins **(de Murcia G., et al., 2004; Bürkle A., 2005).**

The present thesis deals with investigation of properties of matrix-associated PARP, DNA-polymerase β and DNA-topoisomerase II of eukaryotic cells. At present, it is established that PARP is important regulatory protein participates in DNA repair process, cell proliferation, gene expression and differentiation, expression of specific antigens. Such line of investigation is of great importance since recently PARP has been shown to take part in pathogenesis of diseases such as parkinsonism, Alzheimer's disease, diabetes mellitus, myocardial infarction, autoimmune diseases, maliginisation processes and so forth **(Oei S.L., et al., 1997;.Bürkle A., 2005)**. The experiments conducted on DNA-topoisomerase II also seem to be very promising for treatment of different diseases **(Austin A., et al., 2002; Mizutani H., et al., 2002).**

The goal and tasks of the thesis. The work was targeted at studying the influence of ADP-ribosylation on the DNA-topoisomerase II and DNA-polymerase activity and evaluation of effect of different ions and newly synthesized substances on PARP activity of eukaryotic cells. Based on the above discussed facts the following tasks were set:

1. Investigation of the role of ADP-ribosylation in modulation of DNA-topoisomerase II activity in nucleoids of human peripheral blood, mononuclear leukocytes and rat brain cortex neurons.

2. Study of the role of bivalent cations Mg^{2+}, Cu^{2+}, Zn^{2+} and biogenic polyamines (spermine and spermidine) on PARP activity of nuclear matrix of rat hepatocytes.

3. Study of the effect of ADP-ribosylation on DNA-polymerase activity in the nuclei and nuclear matrix isolated from the brain of X-irradiated rats.

4. Evaluation of inhibitory effect of newly synthesized indole cycle containing substances on poly-ADP-ribosylation reaction.

Scientific novelty of the thesis It has been shown possible participation of ADP-ribosylation in modulating the activity of DNA-topoisomerase II localized in the nuclear matrix of eukaryotic cells has via inhibiting action on re-ligation reactioon of the fragmented DNA. Effect of cations Mg^{2+}, Cu^{2+}, Zn^{2+} and biogenic polyamines on PARP activity of nuclear matrix is investigated. Inhibitory effect of newly synthesized indole-cycle-containing substances on PARP activity has been revealed. The new data are obtained which point out that ADP-ribosylation intensifies the DNA repair in the nuclear matrix.

Theoretical and practical importance of the thesis. The obtained results extend knowledge about the nuclear matrix, PAPR, DNA-polymerase β and DNA-topoisomerase II associated with it and seem to be perspective in terms of perfection of treatment of some diseases.

Approbation of the thesis. The results of dissertation have been reported on scientific seminar at the Institute of Molecular Biology and Biological Physics of the Georgian Academy of Sciences.

Publications. 4 articles have been published on the topic of the thesis.

Structure and volume of the thesis. The work is comprised of Introduction, Reference Overview, Results of Investigation, Discussion, Conclusions and List of References, containing 105 titles, is illustrated with 12 figures and 2 Tables. Dissertation is performed on 96 pages.

Matter of the Work

Materials and methods

Nonlinear adult white rats, weighing 150-170 g kept at standard conditions of vivarium served as objects of investigation. Brain and liver tissues were taken as the material of investigation.

Total irradiation of animals was performed by using of an РУМ17 (Russia) apparatus at following conditions: dose power approximately 0.6 Gy/min, current strength 15 mA, voltage 200kV, filters – 1mm Al and 0.5 mm Cu, the skin-focus distance 40 cm. Single irradiation with 4.2Gy dose was applied and the irradiated

animals were decapitated an hour later from the irradiation.

The nuclei from brain and liver tissue have been obtained by centrifugation in high density sacrose solution (Chauveau J., et al., 1956; Заалишвили Т.М., и др., 1989). The preparation of nuclear matrix was isolated after treatment with DNA-ase I using the method of extraction of nuclei with high concentration saline solution (Berezney R., Bucholts L., 1981; Zaalishvili T. M., et al., 2000).

PARP-activity of the nuclei and nuclear matrix preparations was determined by incorporation of adenine ring labeled [^{14}C] NAD into the acid-insoluble product (Заалишвили Т.М., и др., 1980). DNA-polymerase activity of nuclei and nuclear matrix was determined by [^{3}H] dTTP incorporation in acid-insoluble product (Заалишвили Т.М., и др., 1989).

Mononuclear leukocytes were isolated by centrifuging through a Ficoll-400 (ρ=1.078 gr/ml) cusion of fresh venous blood drawn from healthy individuals and stabilized with heparin (Klaus G., 1990).

Fraction enriched (90-95%) with neurons was isolated from rat brain cortex by the methods of Farooq, Norton and Johnson, Sellinger (Farooq M., Norton. W. 1978; Johnson D., Sellinger O. 1971).

The preparations were examined on purity by phase-contrast microscope and number of cells (mononuclear leukocytes and neurons) was counted by Goryaev's chamber.

High-molecular-weight DNA fragments were identified by electrophoresis in pulsating field according to the technique recommended by the producer (CHEF-DR II, Bio-Rad).

Concentration of protein was determined by the method of Bradford (Bradford M., 1976), while that of the DNA was determined by Barton's method (Burton K., 1956) and spectrophotometrically.

Results and Discussion

ADP-Ribosylation Intensifies Cleavage of DNA Loops in the Nuclear Matrix

The architecture of the eukaryotic nucleus includes two overlapping and nucleic-acid containing structures-chromatin and a nuclear matrix. At the third level of chromatin DNA organization, 30nm fibrils are folded in loops with the ends attached to the nuclear matrix. Topologically independent loops vary in size from 5 to 200 kb. Conversion of topological DNA isomers is performed by DNA topoisomerase II, which is associated at the attached regions of DNA loops to the nuclear matrix. DNA topoisomerases functions by coordinately cleaving, manipulating, and religating DNA strands powered by the energy ATP hydrolysis.

The formation of HMW (high molecular weight) DNA fragments is widely thought to result from the excision of DNA loop domains at the positions of their

attachment to the nuclear matrix (Lagarkova M A., et al., 1995; Li T. K., et al., 1999) and is considered to be an initial step in DNA disintegration during apoptosis (Oberhammer F., et al., 1993). Also demonstrated that DNA topoisomerase II is involved in the formation of HMW fragments during apoptosis (Austin A., et al., 2002).

As was mentioned ADP-ribosylation is a reversible covalent posttranslational modification of proteins and is catalyzed in the cell nucleus by PARP. Since various genetic processes are presumably associated with ADP-ribosylation, studies of this modification and its biological role are of great importance. There is evidence that the nuclear matrix, which is a nonhistone protein skeleton of the nucleus, is involved in DNA replication, transcription, and repair (ZaaliSvili T. M., et al., 2000; D'amours D., et al., 1999; Meli E., et al., 2003). Experiments with the purified enzymes have shown that topoisomerase II is ADP-ribosylated and thereby inhibited by PARP (Darby M. K., et al., 1985). In addition, ADP-ribosylation of topoisomerase II has been observed in HeLa cells (Scovassi A. I., et al., 1993).

Based on the above marked we studied the role of ADP-ribosylation in regulating the activity of DNA topoisomerase II in the nuclear matrix of human mononuclear leukocytes and rat brain neurons.

To study the effect of ADP-ribosylation on the nuclear matrix associated DNA topoisomerase activity, matrix preparations were obtained by a recently proposed method (Gromova I.I., et al., 1995). Mononuclear leukocytes and neurons were embedded in agarose plugs. Nuclei of permeabilized cells were treated with a high-ionic-strength buffer to remove topoisomerase II and PARP not associated with the nuclear matrix and to obtain nucleoids containing the nuclear matrix with intact DNA loops. Since matrix topoisomerase II is at the bases of DNA loops, its activity can be detected by DNA cleavage and religation at loop anchorage sites under certain experimental conditions (Razin S., 2001).

ADP-ribosylation and DNA topoisomerase reactions were carried out by incubating agarose plugs in the medium containing 20 mM Tris-Hcl pH~7.5, 50 mM Kcl, 10 mM $Mgcl_2$ and 0.1 mM EDTA, supplemented with 1 mM ATP at 25°C for 40 min. To terminate the reaction and degrade proteins, plugs were transferred into 0.4 M EDTA (pH 8.0), 1% SDS, 0.5 mg/ml proteinase K and incubated in the same solution at 55°C for 36 h. Then plugs were washed with 0.2 M EDTA (pH 8.0) and used for pulsed-field electrophoresis.

The effect of ADP ribosylation on the cleavage of genomic DNA by matrix associated topoisomerase II in mononuclear leukocytes is shown in Fig. 1. The electrophoretic pattern changed when nucleoids were treated with NAD: at 1 mM concentration, NAD stimulats the cleavage of high-molecular-weight genomic DNA. This was evident from an increase in the fraction of 50- to 500-kb fragments and a substantial decrease in the amount of DNA at the start and in the compression zone. The PARP inhibitor thymidine-20 mM (Zaalishvili T. M., et. al 2000) had no effect on DNA cleavage by topoisomerase II in the absence of NAD and completely

abolishes the effect of NAD. This finding indicates that thymidine and NAD do not directly influence topoisomerase II activity and that the activity changes as a result of ADP-ribosylation. Similar results were obtained for neurons (Fig. 2). With the NAD concentration increasing from 0.5 to 1.5 mM, the DNA fraction of 50–150 kb gradually increased and the DNA amounts at the start and in the compression zone decreased. Thymidine (20 mM) abolished the effect of NAD as well as in the case of mononuclear leukocytes, providing additional evidence for the influence of ADP-ribosylation on the topoisomerase activity. It should be noted that similar results were obtained with another PARP inhibitor, 3-aminobenzamide (4 mM),.

DNA topoisomerase II introduces a temporary double strand break in DNA and then religates the DNA ends in the course of topological summarization **(Razin S.V., 2001; Glazkov M.V., 1995)**. It is possible that NAD stimulated DNA cleavage in neuron and leukocyte nucleoids, because DNA religation was inhibited as a result of ADP-ribosylation. This might be caused by modification of DNA topoisomerase II, although we cannot exclude that ADP-ribosylation of another protein(s) affects DNA religation allosterically.

Thus, our results suggest that PARP associated with the nuclear matrix is involved in regulating the activity of matrix topoisomerase II in the eukaryotic cell. Based on mentioned above, it can not be excluded, that at the early stage of apoptosis the formation of high molecular mass DNA fragments (50-500 kb) are going on according to these mechanisms.

Influence of Mg^{2+},Cu^{2+},Zn^{2+} Cations and Biogenic Polyamines on the Nuclear Matrix Poly(ADP-ribose) polymerase activity in the rat liver cells

Metal ions are known to exert influence on chromatin structure while interacting directly with its single components or with chromatin as supermolecular structure. Besides, there is a certain possibility for ions to indirectly act on either structure or function of chromatin **(Zbarsky I. B. 1988)**. Given that perturbation of metal homeostasis accounts for the development of different disorders, study of the metal ions role in the structural – functional organization of eukaryotic genome seems to bear a particular importance **(Thompson K. J., et al., 2001)**.

Along with cations, biogenic polyamines call for no less intensive attention. There are substantial evidences indicating that biogenic polyamines are involved in DNA replication, transcription and protein synthesis **(Gallo C. J., et al., 1986; Blair D.G., 1985; Rodwell V.W., 1996).** It should not be excluded that polyamines play a role in the organization of DNA loop **(Basu H. S., et al., 1993)**. However the influence of metal cations Mg^{2+},Cu^{2+} and Zn^{2+} as well as polyamines (spermine and spermidine) on the matrix PARP activity remains obscure. Based on the above mentioned, we carried out an experimental study of the effects of bivalent cations Mg^{2+},Cu^{2+} and Zn^{2+}

and polyamines spermine and spermidine on the nuclear matrix PARP activity. To approach the target issue in this direction seems correct, since it has been revealed that PARP participates in various genetic processes and is involved with pathogenesis of a number of malignancies, diabetes mellitus, Parkinson's disease etc **(Herceg Z., 2001).**

The trials were conducted using the liver of the rats weighing about 150g. The matrix PARP activity was measured by inclusion the adenine ring labeled [^{14}C] NAD into the acid-insoluble product, while the protein concentration was determined according to Bradford.

Fig. 3 clearly shows that $MgCl_2$ causes a significant rise in the matrix PARP activity. With $MgCl_2$ concentrations of 10 mM and 20 mM there is an approximately 4.2-fold increase in the enzymatic activity. We still do not rule out the assumption that PARP activity might be stimulated by $MgCl_2$ more intensely. The reason may be the $MgCl_2$ –containing buffer solutions employed for the isolation of nuclear matrix preparations, which perhaps, causes the association of Mg^{2+} with the isolated matrix and the increase in the PARP activity in the control preparations.

Regarding the biogenic polyamines spermine and spermidine, they are characterized by an inhibitory effect on the PARP activity. Both spermine and spermidine inhibit enzymatic activity through a concentration dependent way. 1 mM concentrations of spermine and spermidine inhibit the PARP activity by approximately 69% and 67%, respectively Fig. 4. These findings are not consistent with those obtained on the liver nuclear preparation **(Tanigawa Y., et al., 1997)**. It is typical of polyamines to stimulate the PARP activity in the nuclei.

In contrast to Mg^{2+}, Cu^{2+} and Zn^{2+} cations have an inhibitory action on PARP activity depending on concentrations. At the concentration of 1 mM, $CuCl_2$ and $ZnCl_2$ ihibit enzymatic activity by approximately 97% and 93%, correspondingly Fig. 5. It should be noted that $CuCl_2$ and $ZnCl_2$ markedly inhibit the enzymatic activity at a significantly lower concentration (10-20 μM). Identical influence of bivalent cations as well as polyamines also was observed in the case of brain nuclear matrix PARP activity.

Cu^{2+} and Zn^{2+} cations efficiently interact with sulfur-containing ligands. Based on these data, it is suggested thet the cations actively interact with the PARP sulfhydryl groups inducing collectively a decrease in the enzymatic activity.

Thus, the results of the present work show that Mg^{2+},Cu^{2+} and Zn^{2+} cations together with biogenic polyamines represent a group of substances capable of modulating the nuclear matrix PARP activity.

Design of indole derivatives and studying their inhibitory ability of Poly ADP-ribosylation reaction

Over the last few years PARP has been extensively investigated as a target of novel compounds, capable of inhibiting its catalytic activity, that may be used in a broad spectrum of diseases to counteract PARP mediated cell death or to enhance the efficacy of chemotherapy and radiotherapy

At present, it is well established that the enzyme-PARP, important regulatory protein participates in DNA repair process, cell proliferation, gene expression and differentiation, expression of specific antigens.

At 1979 N. Berger suppose that powerful genotoxic stress and high level of DNA damage cause the active response of cells triggering suicide mechanism mediated by PARP. The cell dies before reparation and fixation of damaged DNA, that might lead to the survival of the highly mutated cells **(Berger N. A., et al., 1979)**.

Since 1994 PARP become an object of increased scientific interest because a leading role of enzyme as the mediator of neuronal damage (PARP knockout mice were resistant to neuronal death) **(Zhang J., et.al., 1994)**. Was revealed, that PARP actively participates in the pathogenesis of different diseases, such are neurodegenerative disorders, Alzheimer's and Parkinson's diseases, viral infections, autoimmune diseases, cancer, various inflammatory processes, ischemic diseases and etc. All cases are characterized by the PARP hyperactivation, activation of the poly ADP-ribosylation reaction, irreversible depletion of PARP substrate – NAD and ATP generation processes. It results necrotic cell death. Considering the date mentioned above the scientist are in intensive search of effective PARP inhibitors, which will be capable to reduce PARP activity to therapeutic levels.

It is well known, that indole cycles besides amino acid tryptophan is a part of alkaloids, many of them and their derivatives are biological active compounds and have therapeutic application, but their functioning mechanisms is still to be investigated. The information received in order to solve this problem is rather perspective in terms of revealing the new therapeutic targets.

In the present work we have synthesized compounds containing indole cycles and investigated their inhibitory ability of poly(ADP-ribosyl)ation reaction.

Synthesis of 1,2-dioxo-1,2-dyhidro-α-pyrono[3,2] indole (PI) has been carried out from 6-amino-α-coumarin (1) using Zandmeyer's classic reaction (Fig. 6A), by interaction of (1) with chloral hydrate and hydroxylamine hydrochloride in presence of $Na_2SO_4 \cdot 10\ H_2O$ at 100^0C, during 2 h. Cyclization of obtained product was carried out by 98% H_2SO_4, at 80^0C, 3h. The analog scheme (Fig. 6B) was exploited to 5-methyl-2,3-diketoindolynil (MI) from 4-methylaminophenole via isonitrosoazetanilide, m.p. 184-185^{0}C,

In order to investigate the inhibitory activity of received compounds, the nuclei of rat liver and whole brain were isolated and nuclear PARP activity was

estimated via inclusion of radioactive [^{14}C]NAD labeled in its adenine ring into acid-insoluble product. The reaction was carried out 10 min in the medium (0,2 ml) containing 50 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$,1mM β--mercaptoethanol 50%/DMSO, 0,1 mM [^{14}C] NAD (6,1 mCi/mmol) and 100 μg of protein.

Due to the limited solution ability of MI and MP compounds in alcohol and water DMSO was used. The data on the influence of indol compounds on rat liver nuclear PARP activity is presented in table 1 and Fig. 7A conclusion of the received results is that the inhibitory activity of MI is only 19% Fig. 7A, whenever PI reduce the activity of enzyme by 59% Fig. 7B.

The similar results were obtained for nuclear PARP of rat brain. In this case the inhibitory activity of MI was 20% (Fig.7A) and of PI 60% (Fig.7B). The received results give us the base to suppose certain correlation between the structure of compounds and their inhibitory activity of poly(ADP-rybosyl)ation reaction, that the replacement of methyl hydrophobic groups with aromatic cycle or polar groups that has ability to produce hydrogen bonds contribute to creation of comparatively stable complex of enzyme- inhibitor.

Effect of ADP-ribosylation on endogenous DNA-polymerase activity of nuclear and nuclear matrix of X-irradiated rat brain

As was mentioned above, eukaryotic cell nuclear matrix should take significant part in the processes of DNA replication and transcription. ADP-ribosylation of proteins is a reversible posttranslational modification of proteins and is catalyzed in the cell nucleus by PARP. The latter is supposed to be involved in different genetic processes including excision repair of DNA.

It has been shown in our laboratory that X-irradiation of rats causes redistribution of DNA polymerase β of brain cells from non-matrix sites of the nucleus to the matrix and increase of matrix–associated DNA-polymerase activity **(Заалишвили Т.М и др.,1991).** Our results and the data available in scientific literature **(McCready S. J., et al., 1984)** indicate involvement of nuclear matrix in DNA repair process. Also, ADP-ribosylation has been shown to increase endogenous DNA-polymerase activity in the nuclei isolated from rat brain. Incubation with 1mM NAD of nuclei isolated from rat brain X-irradiated with 1.7 Gy dose 1.5 times increases DNA polymerase activity, while in case of irradiation with 6.7Gy dose DNA-polymerase activity increases 4.7 times. Besides this it has been shown the increase in nuclear matrix-associated PARP activity in irradiation dose depending manner **(Заалишвили Т.М и др.,1989)**, which points to the involvement of PARP in excision repair of DNA.

Considering the facts mentioned above, following experiment has been carried out to reveal participation of ADP ribosylation, proceeding on the level of matrix, in

DNA excision repair. The nuclei were isolated after an hour from the brain of X-irradiated rats weighing 150 g were incubated with NAD (to allow ADP ribosylation reaction to proceed) and deoxyribonucleoside triphosphates (one of them [^{3}H]-dTTP labeled with radioactivity), as a result of which DNA biosynthesis took place (Заалишвили Т.М и др.,1989). After that the nuclear matrices were isolated and inclusion radioactive dTMP was determined on liquid scintillation counter (Заалишвили Т.М и др.,1991).

The experiments have shown that inclusion of radioactive dTMP in the nuclei (isolated from X-irradiated rats-4.2 Gy) incubated with 0.1 mM NAD is nearly 4.4 times higher than in those of nonincubated (Fig. 8A). Incubation of the nuclei with 0.1 mM NAD increases inclusion of [^{3}H] dTMP approximately 8.8 fold in the isolated nuclear matrix (Fig. 8B). 20.9% of the radioactivity inclused in the nuclei was associated with matrix isolated from the nuclei incubated with NAD, while only 10.5% of nuclear radioactivity was linked with the matrix isolated from the nuclei nonincubated with NAD. It should be noted that isolated nuclear matrices containd ~5.2% of nuclear protein and ~2.7% nuclear DNA. PARP inhibitor 3-aminobenzamide (4mM) abolished the effect of NAD, which excludes direct influence of NAD on DNA biosynthesis and provides evidence of influence of ADP-ribosylation on the DNA synthesis. DNA-polymerase-α inhibitor aphidicolin does not affect on inclusion of [^{3}H]-dTMP into acid-insoluble product, as a result provides the evidence of directly involvment of DNA-polymerase-β in DNA repair.

Considering the fact, that chromatin undergoes structural transformations in DNA sites where excision repair proceeds, our results can be interpreted in the following way. Activation of PARP after X-irradiation presumably causes conformational modification of matrix-associated chromatin by ADP-ribosylation which facilitates DNA-polymerase movement on DNA matrix and, correspondingly, is responsible for the increase in DNA biosynthesis.

Conclusions

1. It has been shown using the method of pulse-electrophoresis, that post-translation modification of proteins by ADP-ribosylation in the nuclear matrix of eukaryotic cells presumably have regulating effect on the enzymatic activity of DNA-topoisomerase II, which determines DNA-topology. In particular, it has been shown possible participation of ADP-ribosylation in modulating the activity of DNA-topoisomerase II localized in the nuclear matrix of eukaryotic cells via inhibiting action on religation reaction of the fragmented DNA.

2. It has been revealed that cations of Mg^{2+} considerably increase rat liver nuclear matrix poly(ADP-ribose) polymerase activity, whereas cations of Cu^{2+},Zn^{2+} and biogenic polyamines (spermine and spermidine) significantly inhibit the polymerase

activity in the presence of Mg^{2+} cations.

3. Indole cycle containing compounds 5-methyl-2,3-diketoindolynil (MI) and 1,2-dioxo-1,2-dyhidro-α-pyrono[3,2] indole (PI) capable of inhibiting poly-ADP-ribosylation have been synthesized, on the basis of which more effective compounds of inhibitory nature can be designed in the future.

4. The results obtained point to the participation of ADP-ribosylation in excision repair of DNA. ADP-ribosylation intensifies endogenous DNA-polymerase activity in the nuclei isolated from X-irradiated rats, especially in the nuclear matrix, which indicates the significance of ADP-ribosylation for DNA-repair associated with the nuclear matrix.

The list of publications related to the thesis

1. G. Zaalishvili., N. Japaridze., K. Kolkhidashvili., K. Kutalia., D. Margiani., **Z. Tsetskhladze.,** T. Zaalishvili., Influence of Mg^{2+}, Cu^{2+}, Zn^{2+} Cations and Biogenic Polyamines on the Nuclear Matrix Poly(ADP-ribose) Polimerase Activity in the Rat Liver Cells, ***Bulletin of the Georgian Academy of Sciences, Vol. 170, №1, 2004, pp.169-171***.

2. G.T. Zaalishvili., **Z.R. Tsetskhladze**., D.O. Margiani., I. Yu. Gabriadze., M. Chelidze., T. Zaalishvili. Modulation of the DNA-topoisomerase II Activity by ADP-ribosylation in the Nuclear Matrix of Eukaryotic Cells, ***Proc. Georgian Acad. Sci.,Biol. Ser. B, Vol. 2, No. 5-6, 2004, pp.79-83.***

3. G.T. Zaalishvili., **Z.R. Tsetskhladze**., D.O. Margiani., I. Yu. Gabriadze., T. M. Zaalishvili., ADP-ribosylation Intensifies Cleavage of DNA Loops in the Nuclear Matrix, ***Molecular Biology*** (Moscow), **Vol. 39, No.2, 2005, pp.317-320.**

4. T. Zaalishvili., D. Kharadze., T. Khoshtaria., N. Arabuli., K. Kolkhidashvili., K. Kutalia., T. Omiadze., L. Kirmelashvili., L. Edilashvili., G. Zaalishvili., **Z. Tsetskhladze.,** Design of Indole Derivatives and Studying their Inhibitory Ability of Poly(ADP-ribosyl)ation Reaction, ***Bulletin of the Georgian Academy of Sciences, Vol. 172, №2, 2005, pp.314-316.***