JOINT INSTITUTE FOR NUCLEAR RESEARCH

2009-19

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RESEARCH PROGRAMME OF THE LABORATORY OF RADIATION BIOLOGY: ITS PERFORMANCE IN 2008 AND THE PROGRAMME FOR 2009

Report to the 105th Session
of the JINR Scientific Council
February 19–20, 2009

Dubna 2009

1. Scientific research programme for 2008

The research programme of the Laboratory of Radiation Biology (LRB) determined by the 1st priority theme was concentrated in 2008 on the following main directions: fundamental radiobiological and radiation genetic research with heavy charged particle beams, investigation of molecular photo- and radiobiological processes in eye structures, research in the field of molecular dynamics, radiation research and radiation protection at the basic nuclear facilities of the JINR and its environment.

2. Execution of the 2008 programme

2.1. Radiobiological and radiation genetic research

Complex research on the biological action of ionizing radiation with different physical characteristics has been continued. Regularities of the induction and repair of DNA lesions in peripheral human blood lymphocytes under the action of radiation in a wide range of linear energy transfer (LET) have been studied. Using the DNA comet techniques, regularities have been examined of the formation of DNA single-strand breaks (SSB) and double-strand breaks (DSB) in human lymphocytes under irradiation by ^{60}Co gamma rays and accelerated lithium and boron ions with energy of 40 MeV/nucleon. It has been found that the DNA SSB and DSB yield grows linearly with the dose, the SSB formation efficiency being much higher. The kinetics of the DNA SSB and DSB repair in lymphocytes has been studied during a period of up to 96 hours following irradiation by gamma rays. It has been found that the DNA SSB and DSB yield decreases exponentially in the post-irradiation period and in 24 hours reaches the reference values. This level holds during a further storage of up to 96 hours. A modifying influence has been studied of DNA repair inhibitors – *cytosine arabinoside* (Ara C) and *hydroxyurea* (HU) – on the induction and repair of DNA DSB in human lymphocytes upon irradiation by ^{60}Co gamma rays and accelerated boron ions. It has been found that under the influence of the inhibitors, the number of DNA DSB increases, which seem to be connected with active accumulation of enzymatic DNA DSB. Without Ara-C and HU, the DNA DSB repair is fully completed in 4–6 hours.

Regularities of the induction of different mutation-related lesions by ionizing radiation in *saccharomycete* yeasts have been studied. An analysis of the loss of chromosome IV – one of the biggest chromosomes of yeasts (1554 TPN) – under the action of UV and gamma rays in disomic strains shows that chromosome IV is unstable and is lost more frequently than earlier studied chromosome VII. For example, with the absorbed radiation dose of 100 J/m^2 and cell survivability of ~1%, the chromosome loss frequency is 20×10^{-3}; while for a gamma ray dose of 100Gy and survivability of 10%, it is 4×10^{-3}. A linear dependence of the chromosome loss induction on the dose is observed. The spontaneous chromosome loss frequency is 4×10^{-4}.

The phenomenon of adaptive response (AR) in peripheral human blood lymphocytes has been studied. The main aim of the research was to find out possible reasons for individual variability in the AR of different donors' lymphocytes. A cytogenetic analysis has been performed of chromosome aberrations in human blood upon irradiation by 145 MeV and Bragg peak protons at the therapeutic proton beam of the JINR phasotron. It is shown that the Bragg-peak protons are 1.25 times more efficient in the dose range of 1 – 4 Gy. A comparative analysis has been performed of the individual radiosensitivity of chromosomes 2, 8, and 14 of human lymphocytes to irradiation by charged particles with different LET. At JINR accelerator, healthy donors' whole blood samples were irradiated by accelerated protons and carbon, lithium, and boron ions with the doses of 3, 3.5, and 4 Gy. At the sample locations, the particle energy and LET were, respectively, as follows: 170 MeV and ≈0.5 keV/μm for protons; 480 MeV and ≈10.6 keV/μm for ^{12}C; 30 MeV/nucleon and ≈20 keV/μm for ^{7}Li; 32 MeV/nucleon and ≈55 keV/μm for ^{11}B. A series of experiments were completed at GSI (Gesellschaft für Schwerionenforschung, Darmstadt, Germany) which were performed in collaboration with its Department of Biophysics. The experiments were aimed at studying chromosome aberrations and proliferative activity in human peripheral blood lymphocytes under the action of accelerated iron ions of different energies and LET. To study the human lymphocyte cell response to radiation with different LET, unstimulated isolated lymphocytes were irradiated by accelerated iron ions with energies of 1000 and 200 MeV/nucleon (with a LET of 155 and 335 keV/μm, respectively) and X-rays. Chromosome aberrations and cycle cell progression were analyzed in the first post-irradiation

metaphase cycles 48, 60, 72, and 84 h after irradiation, so practically all dividing cells were included in the analysis. It is shown that after X-ray irradiation, the numbers of aberrations and aberrant cells do not depend on fixing periods. A significant growth in these parameters (two times between 48 and 84 h after irradiation) was observed after irradiation with iron ions with a LET of 155 keV/μm and 8 – 10 times after irradiation with iron ions with a LET of 335 keV/μm. This reflects a time delay in the division of the most heavily damaged cells, which has a pronounced dependence on LET. The effect was yet stronger as LET was higher and ion energy was lower. The coefficients of the relative biological effectiveness (RBE) concerning the chromosome aberration yield varied from 3.0 after 48 hours to 7.0 after 84 hours of cultivation upon irradiation with iron ions with a LET of 155 keV/μm, and, respectively, from 0.5 to 3.0 upon irradiation with iron ions with a LET of 335 keV/μm.

A mathematical model has been developed of the UV irradiation-induced mutation process in the bacterium *Escherichia coli*. It is the first time that on the basis of experimental data, the molecular process has been described that connects the initial stages of the formation of primary DNA lesions with their fixing as mutations. The proposed model concepts have allowed the dynamics of the dimerized products of the *umuD* gene and the main regulatory complexes of the SOS repair system of *Escherichia coli* cells, which has not been studied before, to be predicted.

The developed model concepts have for the first time allowed the dynamics to be predicted of the dimerized products of the *umuD* gene (Fig. 1) and two regulatory complexes of the SOS system: $UmuD_2C$ and UmuDD'C (Fig. 2).

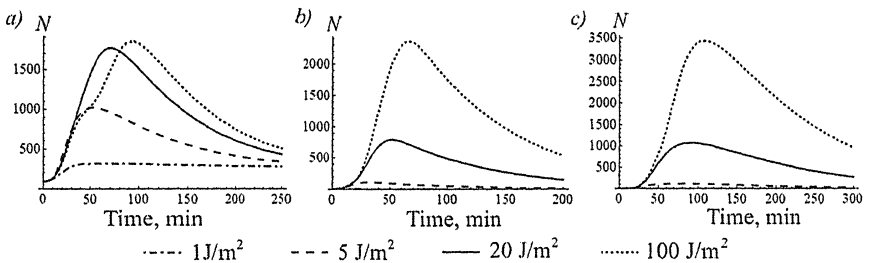

Fig. 1. Numerical calculation of the concentration dynamics of the dimerized protein products of the *umuD* gene for the proteins $UmuD_2$ (a), $UmuD'_2$ (b), and UmuDD' (c).

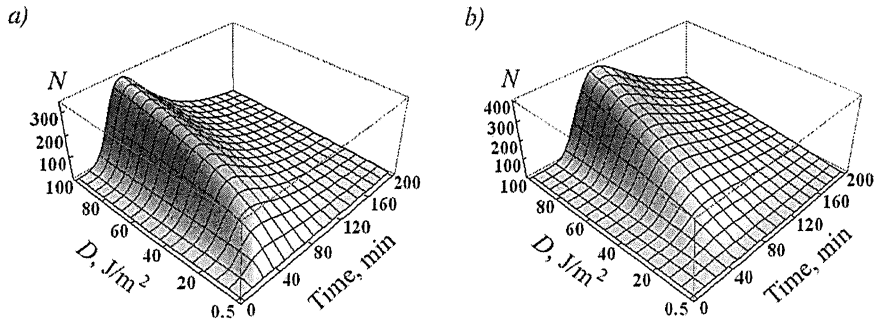

Fig. 2. Numerical calculation of the concentration dynamics of the protein complexes $UmuD_2C$ (a) and UmuDD'C (b).

2.2. Photo-radiobiological research

Research has been continued on the regularities of apoptotic response induced in mouse retina cells by methylnitrosourea (MNU) and ionizing radiation. The research is aimed at further studying *in vivo* the signaling pathway connecting the primary DNA lesions induced by MNU, gamma rays, and protons with mouse retina cell death. It has been found that immediately (no later than two hours) upon introducing MNU, primary DNA lesions are observed as apurinic or apyrimidinic sites (AS), which emerge as a result of the activation of N-glycosylases in the mechanism of the excision repair of methylated bases. Repair of lesions completes six hours after exposure leaving about 50% of initial lesions unrepaired, which remain up to 72 hours. Along with this process, the number of DNA double-strand breaks in retina cells grows monotonically. These breaks are an indicator of the apoptotic degradation of the genome. Their number reaches the maximum in 48 hours. Apoptosis has been confirmed by the analysis of the morphologic changes in the external nuclear layer. But the expression of P53 – a universally recognized regulator in apoptotic signaling in cells – has not been observed. The retina responses to gamma rays (14 Gy) and 170 MeV protons (14 and 25 Gy) were similar: the DNA single- and double-strand breaks induced by these irradiations were efficiently and fully repaired within six hours. Also, in response to proton irradiation, ATM and P53 expression was observed in retina cells in 2 – 4 hours and $\geq$ 12 hours, respectively. No convincing apoptosis indicators have been observed after irradiation. Nicotinamide (Nam) – a PARP-mediated inhibitor of break repair – slowed down DNA repair in the retina after

introducing MNU. But the portion of unrepaired lesions was in this case the same as without introducing MNU – 50%. In this process, apoptosis was fully blocked according to the tissue and cell morphology criteria. A conclusion has been drawn that the retina stability against apoptotic signals is accounted for by the efficient repair of radiation lesions in the transcribable part of the genome of the differentiated cells.

In experiments on C57Black x CBA F1 male mice, research has been continued on the influence of a combination of a single exposure to gamma rays with subsequent daily UV irradiation on a cataract formation. It is shown that on the sixth month after the experiment beginning, weak cortical opacities (1–2 points) develop in all the experimental groups' crystalline lenses. Some of the gamma-irradiated mice also developed posterior polar opacities.

Statistically significant difference in the degree of the crystalline lens damage has been found between the mice that got a dose of 2 Gy and the mice that got a combination of a dose of 4 Gy and UV irradiation ($p=0.015$, the Mann–Whitney test). On the 9th month, the opacity density significantly increased, with the difference in the degree of the crystalline lens damage between different groups being somewhat reduced due to the natural ageing of the mice. In the gamma-irradiated group, the flattening of the epithelium cells and defragmentation of their nuclei has been observed (Fig. 3).

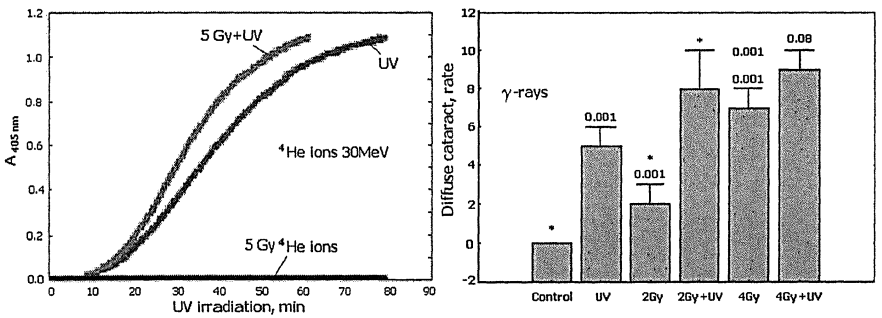

Fig. 3. Study of cataract induction by accelerated heavy ions (*in vitro* - left, *in vivo* - right)

The cell monolayer density was lower for a dose of 2 Gy and much lower for a dose of 4 Gy. On the epithelium layer surface, separate desquamated cells have been observed. Under the action of two damaging factors – gamma and UV radiation – a combination of the mentioned lesions has been observed, but in this case, there were areas of

cubic epithelium transforming into flat one with the formation of two cell layers and areas of capsule detachment from the crystalline lens cell mass. In the case of a combination of a dose of 4 Gy and UV irradiation, a substantial decrease in the epithelium cell density has been observed along with the emergence of cells with large nuclei and sites of epithelium cells transforming into fibroblast-like cells, which formed multi-layer structures. But no significant difference has been found between the groups (the Mann-Whitney nonparametric test). Thus, UV radiation, gamma radiation, and their combination induce changes in the morphology of cataractal crystalline lens cells, but they are not specific to each of the exposures.

2.3. Computer molecular modeling of biophysical systems

Using computer modeling techniques, a comparative study has been performed of the molecular dynamics of rhodopsin containing a chromofore group (11-cis retinal) and free opsin. It is shown that incorporation of a chromofore group into the chromofore center of the opsin has a significant influence on the nearest protein surroundings of chromofore and on the conformation state of the cytoplasmatic domain and has practically no influence on the conformation state of the intra-disk domain. On the basis of modeling results, a possible intra-molecular mechanism of keeping rhodopsin inactive as a G-protein-binding receptor has been considered. A series of works have been carried out to model cyclin-dependent proteinkinases (CDK) with an ATP complex. To analyze the structure changes to which a replacement of CDC28-G20S leads, the crystalline structure of human kinase CDK2 was used. According to the molecular dynamic modeling results, the structures of the non-mutant and mutant (including the replacement of G16S-CDK2 corresponding to the yeast G20S-CDC28) CDK2 complexes notably differ from each other. It is the sites playing the key role in the kinase functioning (for example, the G- and T-loops) where differences between the structural conformations are most apparent.

Molecular dynamics simulations on visual pigment rhodopsin with E181K mutation which is associated with retinitis pigmentosa are performed. Autosomal dominant retinitis pigmentosa leads to the photoreceptor cell death and retina degeneration. Approximately 25% of this pathology are associated with rhodopsin gene mutation RP4(RHO)/Rhodopsin(3q). The amino acid substitution in the

chromophore center during rhodopsin biosynthesis leads to the most distinctive clinical pathology of this inherited disease. The consequence of mutations like these is protein misfolding. As a result, formation of stable Schiff base linkage between 11-cis-retinal chromophore and amino acid residue Lys296 is impossible. Using molecular simulation technique the process of 11-*cis*-retinal chromophore embedding into the chromophore center of opsin mutant form has been investigated. The comparative analysis of amino acid residues arrangement in the opsin chromophore center and its interaction with 11-*cis*-retinal as in the wild (native) as in the mutant opsins has been carried out. It was shown that there are no normal embedding of 11-*cis*-retinal into the chromophore center of opsin mutant form. As a result the impairment of conformation state of the opsin molecule takes place both in the chromophore center and in the cytoplasmic domain. A stable covalent linkage of 11-*cis*-retinal with protein part of rhodopsin molecule is not formed, and also the active site in the cytoplasmic domain of the protein that is responsible for binding of G-protein (so called, transducin) is not completely blocked. Based on the molecular simulation data, the problem related to retinitis pigmentosa pathogenesis is discussed (Fig. 4).

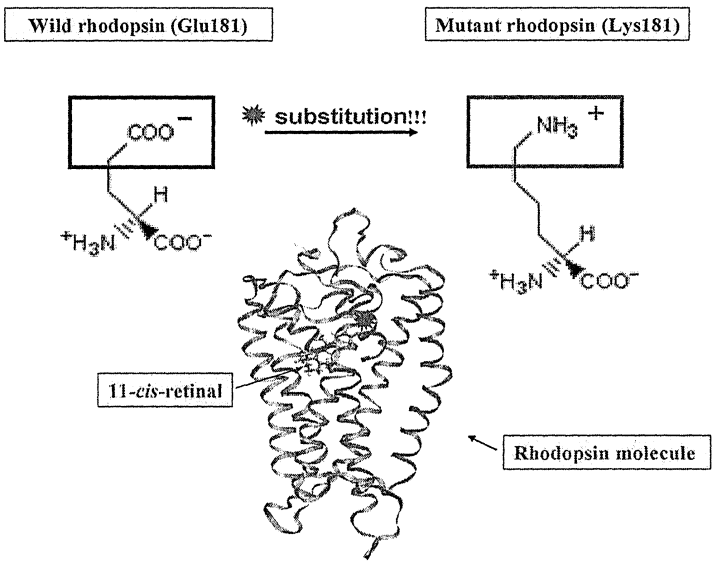

Fig. 4. Mutant form of rhodopsin E181K.

The MD simulation code optimization and performance at different communication architectures for computational chemistry and nanotechnology problems are fulfilled at JINR computing farm, in collaboration with Daresbury Laboratory (UK), RIKEN and Keio University (Japan).

2.4. Radiation research

The works connected with the NICA radiation system design were continued in 2008. The crucial problem determinative the radiation situation around the NICA complex is the problem of "skyshine" neutron dose on the board of the control area where the annual total dose cannot exceed the dose limit for population 1 mSv. All radiation shields at the NICA radiation sources must be designed taking into account this criterion. The most powerful radiation sources at the complex will be the Nuclotron, collider ring and the beam stoppers at the acceleration of the uranium nuclei to energy 4.5 GeV/n. The different variants of the booster, Nuclotron, collider ring shields were simulated by the GEANT4 code for various NICA operation modes. The preliminary verification of the universal MC codes FLUKA, SHIELD and GEANT4 for radiation transport in matter calculation was done as well on the basis of the experimental data on the neutron yields from thick iron target irradiated by ^{238}U nuclei with energies 1 GeV/n. The technical draft proposal for the radiation shielding design was prepared.

The investigations connected with the development, computer modeling and physical calibration of the Russian neutron detectors DAN (Dynamic Albedo Neutrons) and LEND (Lunar Exploration Neutron Detector) assigned for the Mars and Moon surfaces researches by the nuclear-physical methods were continued in collaboration with the FLNP and the Institute of Space Research (Moscow). The setup works for the LEND is now practically completed before the space vehicle launch. The DAN calibration was done while the pseudo environmental tests and shown the high sensitivity of the detector for the underground water (ice) seek by the NACA rover (Mars Science Laboratory).

The work within the framework of project "Development of new protection materials and thermo luminescent detectors for radiation safety measures" of the complex long-term cooperation program between Russia and India was continued. The experiments were performed at a 150 MeV proton beam on studying the properties of several tens of

thermo luminescent phosphors fabricated in India using nano- and micro technologies. The other experiments on studying the properties of protection materials fabricated in India were carried out as well. The simulation of the ridge filter assigned for the carbon therapy installation was done by the MC code.

3. Scientific research programme for 2009

The central problem of fundamental radiation-genetic investigation is the clarification of molecular mechanisms of the mutagenic effects in cells with various levels of genetic organization due to different types of ionizing radiations. Heavy charged particles are very powerful tool for investigation of mechanism induction of radiation-stochastic effects. The character of the DNA damages induced by the charged particles irradiation (particularly by heavy ions) is strongly differed as compared with gamma exposure. One of the specific characteristics of genetic effects of heavy charged particles is the induction of clustered DNA damages. Such lesions are the result of significant energy deposition in genetic structures. In this case the repair of single and double strand breaks of DNA may be strongly suppressed. The consequence of it reflects on very serious genetic effects. The specificity of heavy ions biological action is so important that it allow one to speak about the "new radiobiology" as against of "classical radiobiology". The new line of the radiobiological research at the cellular level will be connected with application of the confocal CARS microscope, which will set in operation at the LRB in 2009. The operation of the microscope is planned from 2010. The other applications of CARS microscope will be concerned with investigation of the nanostructure objects in collaboration with LHEP and LNP.

Research and development will include:

- Study of DNA molecular damages of human cells induced by heavy charged particles and reparation of molecular in human cells induced by radiation with different LET;
- Study of mechanisms of a cancer formation and risk estimation of occurrence of chronic myelome leucemia at persons exposed by small doses of ionizing radiation;

- Investigation of peculiarities and mechanisms of stable and unstable chromosome aberrations induction in human cells, point and structural mutation induction in low eukaryotic cells by irradiation with heavy charged particles;
- Investigation of effects of low doses radiation with different LET on the chromosomal apparatus of cells and problem of cell recovery;
- Cognition of the mechanisms determined cell's hyper sensibility and radioresistance at low doses of radiation;
- Study of damaging effects of heavy charged particles on human eye retina and rodopsin;
- Computer molecular modeling and simulation analysis of biological structures;
- The estimation of biological efficiency of therapeutic proton and carbon beams on the human cells through cytogenetic consequences of peripheral blood lymphocytes;
- Theoretical modeling of ionizing radiation interaction with matter (including interactions with biological structures), shielding and activation calculations;
- Prognostication of radiation situation at the NICA, large experimental installations and spacecraft boards;
- Investigation in the field of targeted cancer therapy.

As the results the following items will performed:

- Data on induction of double strand breaks of DNA in human blood lymphocytes at action of heavy charged particles;
- Data on regularities of unstable chromosome aberrations forming due to action of low doses of radiation;
- Data on mechanism of mouse eye cataract induction at action of protons with energies 100-200MeV;
- Modeling of mutagenic process in *E.coli* cells irradiated by UV-rays;
- Radiobiological experiments at LHEP nuclotron, LNR cyclotron and LNP phasotron;
- MD simulation of the main biomolecular structures;
- Radiation shields estimation for the NICA project;
- Data on influence of SHF electromagnetic radiation on heavy metal nanoclustrers incorporated into biological structures.

4. Scientific meetings and educational activity

The 3rd international workshop "Molecular Simulation Studies in Material and Biological Sciences" (MSSMBS'08) was held on 10-12 September at JINR. Scientists from the research centers and universities of Japan and Europe, leading research centres of Russia (Institute of Bioorganic Chemistry, Institute of Biochemical Physics, Institute of Mathematical Problems of Biology, MSU) as well as from the LRB and LIT attended the workshop. The workshop scientific programme reflected the today status and perspectives of the computer molecular simulation in the modern science. The MSSMBS-2008 scientific program has covered the following topics: protein modeling, drug design, simulation of liquids and polymer systems, simulation of radiation-induced damages and mutations, quantum biophysics, parallel computing for chemical physics and biomolecular studies.

The education process at the chair "Biophysics" of the International University "Dubna" was continued. 76 students in sum are studying now on specialty - "Radiation protection of people and environment" and 17 new students were admitted in 2008 to the chair. The 4th graduation of the 7 students took place in 2008.

5. Administration activity

Personnel. The total personnel of the LRB were 85, including the directorate staff 8.

Finance. Funding of research in the direction of radiation and radiobiological investigations in 2009 is shown in Table 1.

Table 1. Financing LRB in 2009.

Area	Financing plan (k$ USA)
04-9-1077-09/2011 (1st priority)	674.0
Infrastructure	179.7
Total	853.7

Отпечатано методом прямого репродуцирования
с подготовленного лабораторией оригинала.

Получено 05.02.2009. Подписано в печать 09.02.2009.
Формат 60 × 90/16. Бумага офсетная. Печать офсетная.
Усл.-печ. л. 1,18. Уч.-изд. л. 1,26. Тираж 105 экз. Заказ № 56496.

Издательский отдел Объединенного института ядерных исследований
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