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Original research paper

MUTAGENIC EFFECTS INDUCED BY ACCELERATED ^{11}B IONS WITH ENERGY OF 12-34 MeV/n ON YEAST *SACCHAROMYCES CEREVISIAE*[*]

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Abstract. *In connection with the active space exploration, the studies of the effects of heavy ions are currently of particular interest. Cosmic radiation is primarily composed of protons and other ions with energies of >10 MeV. We have modulated the effect of cosmic radiation by using heavy-ion beams at the Heavy-Ion Accelerator in Dubna (JINR). Particularly, we have investigated the biological effects induced by accelerated boron ions with the energy of 12-34 MeV/n and linear energy transfer (LET) 42, 61 and 101 keV/μm. Dose dependence of lethal damage, the induction of point mutations and chromosome rearrangements were studied. The effectiveness increased with the increase of LET and maximum effectiveness occurred at 61 keV/μm for inactivating and mutant effects.*

Key words: *Accelerated boron ions, gene mutation, frameshift, base pair substitution, deletion, yeast* Saccharomyces cerevisiae

Densely ionizing radiations such as are found in cosmic radiations or are obtained from heavy-ion accelerators are of interest to radiobiologists because of differences in their action on biological objects compared with action of rarely ionizing radiations. Determination of relative biological effectiveness (RBE) of radiations as a function of linear energy transfer (LET) is necessary for the understanding of molecular nature and repair of radiation-induced genetic damages. It may be studied at the level of primary lesions (single-strand break, double-strand break, complex lesion) or realized mutations. This problem was formulated in 1950s-1960s of the XX century [1, 2] but may be resolved and developed now by modern methods. Unicellular eukaryotic yeast has been subjected to extensive radiobiological and genetic investigations that provided a suitable basis for such investigations. We selected advanced genetic systems to study the different effects (lethality, frameshift mutation, base pair substitution, chromosomal and plasmid rearrangements). A forward mutation rate assay detects mutations inactivating the arginine permease gene (Can^R mutations), frameshift reversion assay detects mutation that reverts a 4-base insertion in the *LYS2* gene (*lys2ΔBgl*) [3], and special *trp1*-reversion assay detects transition GC-AT [4]. Intrachromosome homologous recombination assay consists of two mutant alleles *lys2* located in chromosome II (first — the 5'trancated *lys2* sequence and the *LEU2* gene integrated into chromosome II as a direct repeat, second — the *lys2::HS-D* allele which is 658 bp-insertion in *BamH1* site of 3'-termini of *LYS2* gene) [5]. The [YCpL2]-plasmid assay was used to detect the extent deletions including two or more genes [6]. Earlier we represented the results of irradiation of yeast cells by protons [7]. In present work we represented the results of irradiation of yeast cells by accelerated boron ions.

The source of γ-rays was ^{60}Co (the dose rate 0.7 Gy/min, a LET of 0.25 keV/μm) at therapeutic equipment "Rokus" (JINR, Dubna). Cells were irradiated in Eppendorf tubes and kept in ice for prevention of DSB repair. Boron ions irradiations were carried out at the Heavy-Ion Accelerator U-400M in Dubna (JINR). The energies were 12, 22, 34 MeV/n (LET of 101, 61, 42 keV/μm). The dose rate was 0.55 Gy/min. The yeast cells were irradiated with boron ions at a dose up to 100 Gy. The total range of 30-MeV boron ions is more than 100 μm in cell culture. The mean LET of the radiations studied did not vary appreciably throughout the target culture. Small size of cells (5 μm in diameter) appears to irradiate a large number of the cells in thin layers of cultures. This is an advantage in obtaining statistically significant results. Overnight cultures (~2x10^8 cells/ml) were grown in YEPD. The percentage of budding cells was less than 3 %. The cells were centrifuged, washed, and resuspended in water to a concentration 10^9 cells/ml. For irradiation, dry 4 % agar in case were prepared and covered by mylar film with a diameter of 20 mm. 100-μl samples were pipetted onto the films. Cases were kept on ice before and after irradiation. Immediately before irradiation, cases were fixed on a cassette disk.

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Beam monitoring and automatic change of cell patterns nested on the disk container were provided by the special electronic equipment. After irradiation, the cells were resuspended by transferring each film to a tube containing 2 ml of sterile water.

Irradiated cell suspension, after serial dilutions, was plated on appropriate SM-based selective media or on YEPD to assess mutagenesis and cell survival, respectively. Selective growth was on synthetic complete medium containing 2 % glucose (SM) [8] and lacking the appropriate nutrient SM-Trp, SM-Lys [9]. Canavanine-resistant mutants in the forward mutation assay were identified on SM-Arg plates supplemented with 60 µg/ml canavanine. All growth was at 30° C. Colonies arising on YEPD and appropriate selective media plates were counted after 3-5 or 5-7 days of incubation, respectively.

The study of the biological effect of accelerated boron ions irradiation showed that cell survival was exponential for all used strains (Fig. 1). The RBE of cell inactivation increased with the increase of LET. For the used strains, the average value of RBE for the accelerated boron ions with the energy of 34 and 22 MeV/n (LET 42 and 61 keV/µm) was 1.8±0.5 and 2.8±0.5, respectively. These are in good agreement with the earlier results obtained by Tobias [1] with haploid *Saccharomyces cerevisiae*. It was shown that at low LET the radiosensitivity was approximately constant, then, there was a region of increasing sensitivity with increasing LET and finally, at high LET values, there was a region of decreasing sensitivity. The observed maximum RBE of ~2.0 was obtained at LET 120 keV/µm.

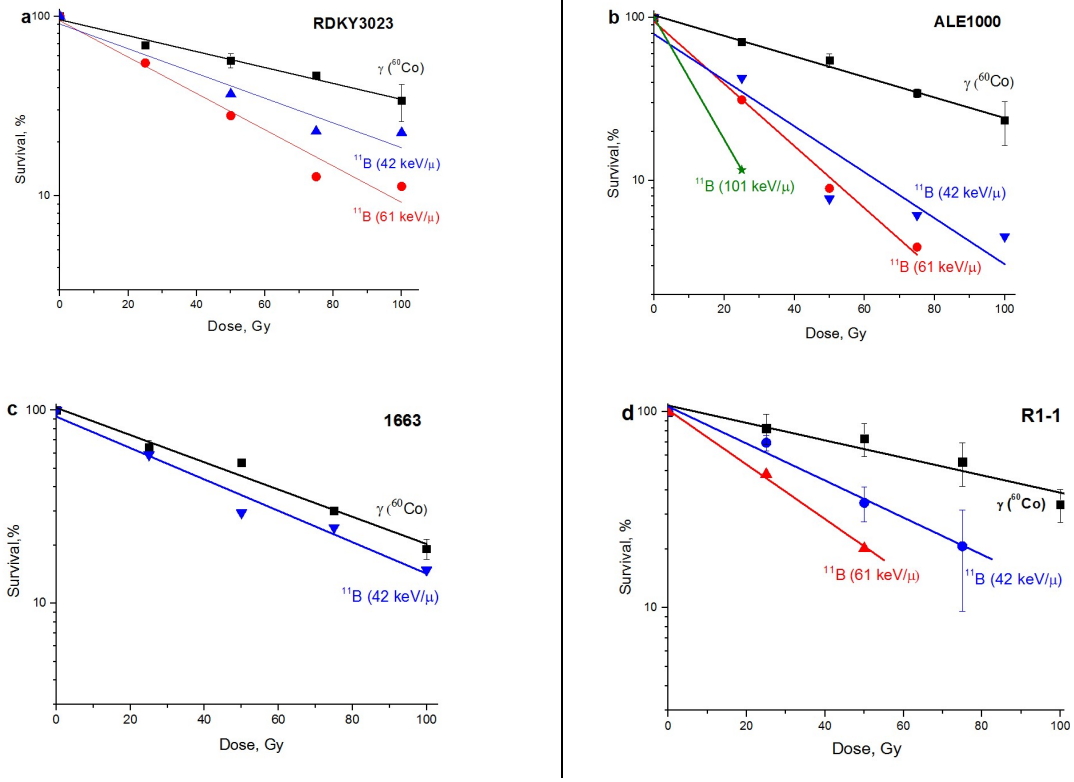

Figure 1. Survival of the haploid yeast strains RDKY3023 [3] (a), ALE1000 [5] (b), 1663 [4] (c) and R1-1 (d) exposed to γ-rays (three experiments) and accelerated boron ions with LET 42, 61 and 101 keV/µm (one-two experiments per each LET)

The frequency of point mutations (forward, frameshift, base pair substitution) and ectopic recombination were studied for γ-ray and boron ions irradiation. For each curve on Fig. 2, the coefficient of determination R^2 and fitting function (pol - polynomial, exp – exponential) were calculated. R^2 of gene mutations was 0.94-0.999 but the fitting of chromosome rearrangement dependence was difficult. The mutation induction curves for these radiations were described by the linear-quadratic curves for γ-ray irradiation and by polynomial or exponential curve for heavy ions irradiation. But, in several cases, the dose dependence was linear, for example, for such rearrangement of DNA as large deletion in plasmid. The boron ions were more effective in the induction of deletion mutations than the γ-rays. However, other results were obtained for diploid yeast [2, 10]. Reversion induction curves and frequency of crossing over were linear up to 150-200 Gy. Beyond this dose, the mutation induction curves in some cases turned upward and the reproducibility between experiments was poor. (In our experiments with haploid strains this border occurred earlier at ~100 Gy.) Crossing over the over-all relation with dose was complex and went to plateau. The same results were obtained for bacteria for which these dependences were studied well. The frequency of mutations as a function of γ-ray and heavy ions dose was described by the linear-quadratic curves

in the case of point mutations and the linear function in the case of deletion mutations [11]. But the frequency of the Tn10 precise excision was exponential function from the irradiation dose [12].

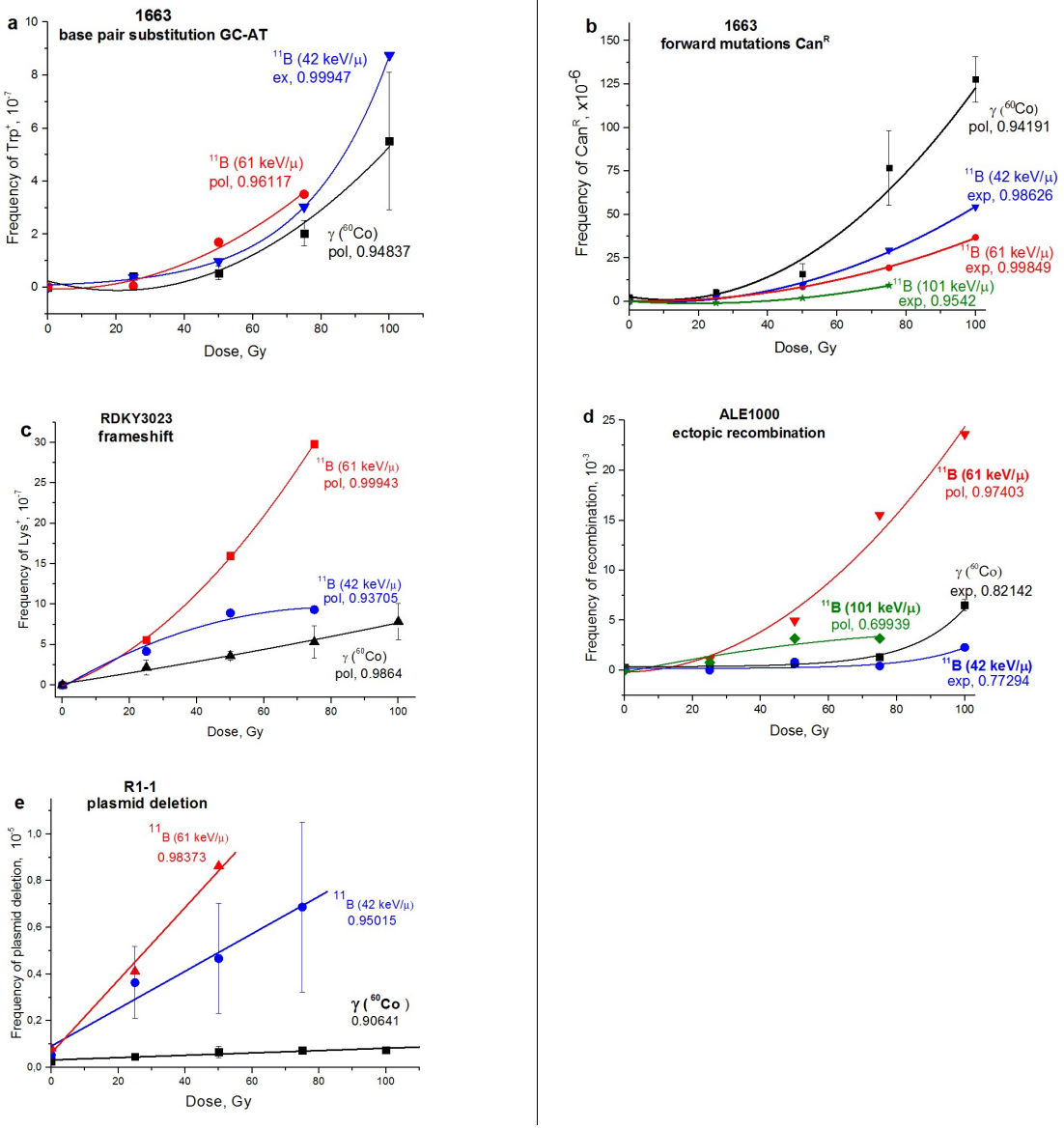

Figure 2. Frequency of base pair substitution GC-AT (a), forward mutations Can^R (b), frameshifts (c), recombinations (d) and plasmid deletions (e) induced by irradiation of γ-rays and accelerated boron ion with LET 42, 61 and 101 keV/μm.

It was known that for bacteria with an increase in LET, the RBE coefficients of heavy ions estimated on the basis of their lethal action, gene and deletion mutation induction increase [10, 11]. The RBE depends on LET as a function with a local maximum but the dependence RBE from LET was different for different types of events. For bacteria the LET value, to which maximal RBE coefficients correspond, varies depending on the character of the observed radiation-induced effect — 20 keV/μm for the gene mutations, 40 keV/μm for deletion mutations, 20-40 keV/μm for Tn10 excision and 100 keV/μm for the lethal effect. The maximal RBE also depends on the character of the observed radiation-induced effect —~2 for the gene mutations, ~3 for deletion mutations and ~2.5 for the lethal effect.

In the case of eukaryotic yeast cells, the RBE depends on LET also as a function with a local maximum (Fig. 3). The maximum of RBE also depends on the nature of events. For lethal effect, the maximum of RBE (~3) corresponds to LET ~80 keV/μm. For ectopic recombination and deletion in plasmid the maximal RBE (~2 and ~7) corresponds to LET ~61 keV/μm. The point mutations had maximal value (~2) for base pair substitution and frameshift mutations. The strange results we obtained in the case of forward mutation (Can^R). It had the plateau followed

by a decrease. These results need to be confirmed by additional experiments.

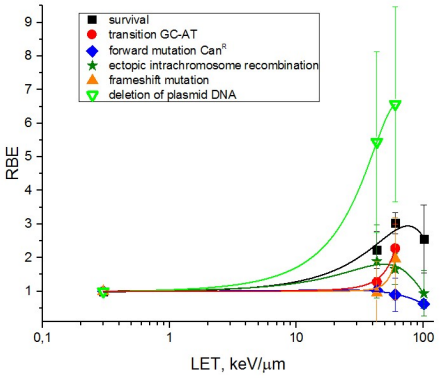

Figure 3. RBE versus LET for different mutations and inactivation of haploid yeast *Saccharomyces cerevisiae*

However, for diploid yeast cells, for all effects, the maximum efficiency was observed at 125 keV/μm [2]. RBE of inactivation, recombination and gene mutations were ~3, 2.5 and 2 respectively. For mammalian cells, the RBE-LET dependence of heavy ions was also described by the curve with a local maximum: for survival it was at ~80-200 keV/μm and for mutation induction – at ~80 keV/μm [13].

Summary. This investigation had shown an increasing mutagenic influence of radiation on haploid yeast cells in the LET region up to 100 keV/μm. The heavy ion irradiation with a LET 60 keV/μm turned out to be most efficient for wide spectrum of mutations. Differences in the RBE occur for various effects. The relation between RBE and LET was the same for haploid/diploid yeast and mammalian cells. However, the dose dependences were different for them and reflect the differences of molecular mechanisms of induction of these effects.

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