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Plant radioresistance and DNA repair efficiency in *Chlamydomonas reinhardtii* and *Pisum sativum*

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Abstract

This paper compares the repair of DNA single strand breaks (ssb) induced by γ-radiation in two strains of *Chlamydomonas reinhardtii* (137C/+/ and UVS-I) and three lines of *Pisum sativum* (NN 131, 198, 140) differing in the degree of radioresistance. DNA ssb in cells exposed to γ-rays (50, 100, 200, 500 Gy) were measured by electrophoresis and alkaline unwinding method with subsequent chromatography on hydroxyapatite immediately after irradiation and after 30 min of post-irradiation incubation at 25 °C. An increase of double-strand DNA (in %) was found in cells after 30 min post-irradiation incubation. *C. reinhardtii* strains displayed an equal level of DNA degradation and repair efficiency in the DNA single strand breaks. The radioresistant line N 198 of *P. sativum* is characterized by a lower level of induced DNA ssb and higher efficiency of repair of these breaks as compared with less radioresistant lines NN 131 and 140.

Introduction

At the present radioresistance of an organism or a cell is considered to depend mainly on the properties of its DNA repair systems (Boreham and Mitchel 1991, Chankova *et al.* 1990, Fox 1990, Kaina *et al.* 1990, Sergeeva *et al.* 1984, Wheeler *et al.* 1988, Wlodek and Hittelman 1987, Yasuhira *et al.* 1991). A correlation between cell survival and efficiency in the repair of DNA single strand breaks (ssb) was obtained in the Chinese hamster ovary cell lines irradiated with UV-A radiation (Churchill *et al.* 1991). A number of radiosensitive mutants of bacteria, yeast and mammalian cells have reduced levels of DNA ssb and repair of double strand breaks (dsb) (Fuller and Painter 1988, Sargentini and Smith 1986, Thompson *et al.* 1982). On the other hand the initial yield of ssb DNA and the rates of their repair was the same in murine leukemic lymphoblast cell lines L 5178 Y-R (resistant) and L 5178 Y-S (sensitive). In this case the DNA dsb were suggested to be an important factor

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determining the radiosensitivity of L 5178 Y-S cells (Wlodek and Hittelman 1987). Some investigators also showed a correlation between radiosensitivity to γ-rays radiation and the ability to repair DNA dsb (Eguchi-Kasai *et al.* 1991, Kelland *et al.* 1988). On the other hand no correlation between cell lethality and slowly repairable DNA strand breaks, probably dsb, was found (Sakai and Okada 1984). Hanawalt (1991) suggested that overall genomic DNA repair efficiency did not necessarily correlate with cellular sensitivities to radiation and other DNA damaging agents.

Therefore we studied induction and repair of ssb in lower (unicellular green alga *Chlamydomonas reinhardtii*) and higher (*Pisum sativum*) photosynthetic organisms with different radioresistance.

Materials and methods

Pisum sativum mutant lines N 140, N 198 and control line Sofia 131 were used in this work. The induction and genetic characteristics of these mutant lines were described previously (Mehandjiev 1972). The radioresistance after γ-radiation (^{60}Co, I = 12.5 rad s^{-1}, dose 100 Gy) was assessed according to germination and plant survival in laboratory and greenhouse conditions. 120 - 150 seeds of *Pisum sativum* lines were sterilized for 5 min in a mixture of ethanol and 3 % H_2O_2 in ratio 1:1. The seeds were cultivated in sterile Petri dishes on filter paper, saturated with liquid nutrient medium of Tamiya (Vaulina *et al.* 1978) diluted 10 times, under continuous light at temperature 25 °C for 7 d. The germinated plants were divided into 3 parts (25 plants for each variant): control non-irradiated plants, held on ice for a time equal to the irradiation plus incubation times; irradiated plants-held on ice for a time equal to the incubation time and irradiated plants incubated at 25 °C for 30 min. The green parts of the plants (stems and leaves) were used for DNA analysis in denaturating gel electrophoresis and the roots for determining the DNA single and double fragments using the specific reaction with 3,5-diaminobenzoic acid.

Chlamydomonas reinhardtii strains 137C/+/ and UVS-I were supplied from Prof. Tugarionov's Laboratory, Sanct-Petersburg State University, Russia. The UVS-I strain was chosen because the cells were deficient in dark repair (Small 1987). The algae were grown in continuous light on synthetic medium of the following composition (per dm^3): 0.15 g NH_4NO_3, 0.02 g $MgSO_4.7H_2O$, 0.01 g $CaCl_2.2H_2O$, 0.363 g KH_2PO_4, 0.737 g K_2HPO_4, and 1 cm^3 trace element solution (Harris 1989). The suspension was in a stationary phase condition at the end of incubation period (5 d). Cells were isolated by centrifugation, washed and resuspended in the fresh growth medium to the cell density of 4 - 5 × 10^7 cells cm^{-3}. The cell suspension was split into 3 parts: control, irradiated cells and irradiated cells incubated at 25 °C for 30 min. ^{137}Cs γ-rays source (*GUPOS* - 4.2 Gy min^{-1}, doses 50, 100, 200, 500 Gy) was used. Strain radioresistance was evaluated by colony-forming ability (Shevchenko 1979).

Detection of ssb DNA and determination of their repair efficiency: The efficiency of formation and repair of DNA ssb induced by acute γ-radiation of 50 and 200 Gy were determined in algae and pea growing under laboratory conditions. Number of DNA breaks was determined by means of denaturating gel electrophoresis of single-strand DNA and by the alkaline unwinding method with subsequent chromatography on hydroxyapatite (Rydberg and Johanson 1975). The quantity of double strand (ds) and single strand (ss) DNA was determined by reaction with 3,5 diaminobenzonic acid (Kissane and Robins 1985).

Isolation of DNA: Samples, homogenized in liquid nitrogen were suspended in five volumes of lysis buffer (0.1 M Tris/HCl, 0.05 M EDTA, 2 % SDS, 0.1 % mercaptoethanol, 0.4 % polyvinylpyrrolidone, pH 8.0) and incubated at 60 °C for 10 min. Sodium hypochlorite (5 M) was added to the homogenate, and the mixture was incubated for 10 min under gentle stirring followed by deproteinization with phenol and chloroform (1:1). RNase treatment and sedimentation with ethanol were carried out according to conventional techniques (Maniatis *et al.* 1982). At the final stage DNA was diluted in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) to a concentration of 60 -100 μg cm^{-3}. For denaturation, an equal volume of 10 M urea was added to the DNA solution and the mixture was heated on a water bath at 95 °C with subsequent rapid cooling in ice-cold water. Electrophoresis was carried out in a horizontal gel box at 150 V for 2 h with ultrathermostat water cooling. The electrode buffer consisted of 0.04 M Tris-acetate and 0.002 M EDTA, pH 7.6. Agarose gel (0.7 %) was prepared on the electrode buffer with the addition of 1 M urea. The gel was stained with etidium bromide (5 μg cm^{-3}) in 1 mM $MgSO_4$ for 1 h. λ-phage DNA, restricted with Bam H I and Pst I was used as a relative molecular mass marker. Gel negatives were cut along separate lanes and scanned in a *Specord M 40* (*Carl Zeiss*, Jena, Germany) spectrophotometer. The distance covered by the DNA maximum was determined in the same way as the mean numerical DNA mass and the quantity of ssb according to Yamamoto *et al.* (1982). The data presented are based on three repetitions for each variant. For all tests divergence from the control is significant at $P < 0.01$.

Results

Radioresistance: *Pisum sativum* mutant line N 198 expressed the highest radioresistance according to germination and survival criteria. There was no significant difference in radioresistance between the control line N 131 and the mutant N 140 (Table 1). In *Chlamydomonas reinhardtii* there were no significant differences between cell survival for strain 137C/+/ and UVS-I in a dose range from 50 to 200 Gy (Fig. 1).

DNA ssb induction and repair efficiency: Lower level of ssb was induced in the most radioresistant mutant line N 198 than in the other *Pisum sativum* lines (Table 1). At the same time enhanced ssb DNA repair efficiency was found after 30 min post-irradiation incubation when compared to control line N 131. Control line N 131 and

mutant line N 140 demonstrated approximately equal level of radioresistance according to the plant survival. This sensitivity did not correlate to ssb level and ssb DNA repair efficiency. Mutant line N 140 showed higher level of ssb as well as lower level of ssb DNA repair efficiency than control line N 131.

Table 1. Radioresistance of *Pisum sativum* lines against γ-radiation (100 Gy).

Radioresistance criteria	*Pisum sativum* lines N 131	N 140	N 198
Seed germination [%]	64.0 ± 1.23	60.0 ± 1.26	96.0 ± 0.50
Plant survival [%]	61.0 ± 1.25	57.0 ± 1.27	95.0 ± 0.56
ssb level Rad/Da	5.9×10^{-12}	8.6×10^{-12}	1.4×10^{-12}
Repair efficiency [%]	66.8 ± 4.15	44.6 ± 4.55	92.9 ± 5.25

Data represent the mean of three independent experiments ± S.E. Differences from the control are significant at $P < 0.01$.

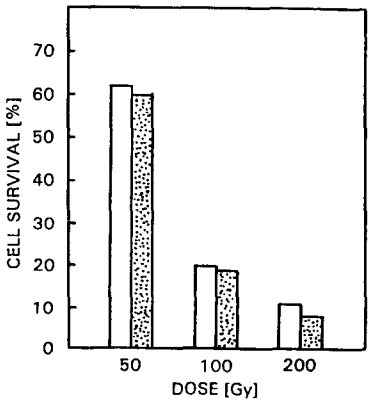

Fig. 1. Effect of γ-radiation on colony-forming ability in strain 137C/+/ (*open columns*) and UVS-I (*closed columns*) of *Chlamydomonas reinhardtii*. Means of three independent experiments; differences from the control (= 100 %) are significant at $P < 0.01$.

Table 2. Induction and repair efficiency of DNA ssb in *Chlamydomonas reinhardtii* strains after γ-radiation (500 Gy).

Strain	DNA ssb level Rad/Da	Repair efficiency [%]
137C/+/	3.0×10^{-12}	86.35 ± 2.45
UVS-I	3.4×10^{-12}	83.55 ± 3.55

Means of three independent experiments ± S.E. Differences from the control are significant at $P < 0.01$.

Both *Chlamydomonas reinhardtii* strains displayed approximately the same level of ssb induction. An increase of double-strand DNA was found (Fig. 2) after 30 min post-irradiation incubation at 25 °C in cells exposed to γ-rays. Strain UVS-I showed no significant difference from wild type 137C/+/ in ability to rejoin ssb. Analogous results were obtained by the method of electrophoresis (Table 2). There were no differences between ssb DNA level induced by γ-radiation (500 Gy) for both strains and these strains expressed similar repair efficiency, too.

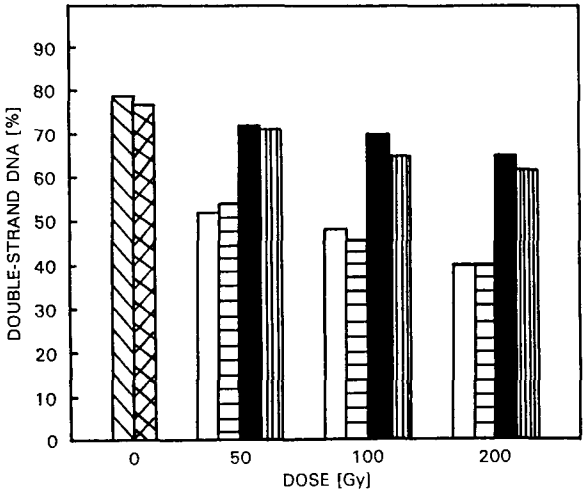

Fig. 2. Double-strand DNA in control non-irradiated strains (137C/+/ - *hatched column*, UVS - I - *chequered column*), immediately after γ-irradiation (137C/+/ - *open columns*, UVS-I - *horizontally hatched columns*) and after 30 min post-irradiation incubation at 25 °C (137C/+/ - *closed columns*, UVS-I - *vertically hatched columns*). Means of three independent experiments. Differences from the control are significant at $P < 0.01$.

Discussion

Our results showed no significant difference in radioresistance between UVS-I and wild strains of *Chlamydomonas reinhardtii*. Similarly population of cells of the UVS-I mutant had survival curves after γ-radiation similar to those of wild-type cells (Harris 1989). Also ssb DNA damages for both strains and ssb DNA repair efficiency after incubation were similar. This tendency was confirmed by both methods used. It is possible to suggest that strain UVS-I possesses ability to repair DNA ssb induced after γ-radiation regardless of the fact that this strain is described as dark repair deficient strain in the case of UV-irradiation (Harris 1989). Probably elevated UV-sensitivity of UVS-I strain is caused by the disturbance in the excision repair of dimers, in particular the activity of a specific enzyme UV-endonuclease.

On the other hand *Pisum sativum* lines with various radioresistance differed in their ability to form ssb and/or ssb DNA repair efficiency. Results showed that the radioresistance of *Pisum sativum* mutant N 198 correlated well with the lower level of ssb and higher level of ssb DNA repair efficiency. So the evidence to support the

existence of efficient ssb DNA rejoining in a radioresistant mutant of *Pisum sativum* was found. It could be that ssb induction and repair efficiency are one of the mechanisms taking part in the formation of increased radioresistance. However, no correlation was found between radiosensitivity to γ-rays and the repairability of induced ssb for both lines N 131 and N 140.

Our results are in agreement with other data showing that different organisms may have various radioresistance because they differ in the spectrum and the quantity of specific lesions produced in their DNA (Teoule and Cadet 1978, Von Sauntag and Schulte-Frohlinde 1978), the rate, extent, distribution and fidelity of their DNA repair (Ward 1981, Goodhead 1985, Wheeler and Nelson 1987) and their physiological state (Wheeler 1987).

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