

γ - Irradiation Effect: Variation of Photosynthetic Activity of *Euglena*

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Objective To study the effects of gamma-ray irradiation on carbon fixation (Specific production rate: SPR), CO₂ utilization efficiency (CUE) and electron transfer rate (ETR) in the photosynthetic flagellate *Euglena gracilis* strain Z in a dose-response dependent manner. **Methods** *Euglena* cells were cultured in an inorganic nutrient medium containing ammonium chloride or proteose peptone. Cells were exposed to gamma-ray at 5 doses (0, 100, 250, 350, 500 Gy for water). Five days after irradiation, three photosynthetic activities were measured. SPR, which is a carbon uptake rate per unit carbon mass, was determined by ¹³C tracer methodology. CUE was evaluated using a relation of carbon isotope fractionation in Calvin cycle. ETR in photosystem II (PS II) was measured by a chlorophyll fluorescence analysis. **Results** Even at a dose of 500 Gy, 80 % of ETR of the non-irradiated control (0 Gy) was sustained, while SPR and CUE were about half the level in the non-irradiated control at 500 Gy. Furthermore, the dose response of ETR was considerably different from the others. **Conclusion** Our findings suggest that not only PS II but also the Calvin cycle in the photosynthetic system is affected by gamma ray irradiation.

Key words: Gamma-ray irradiation; *Euglena gracilis*; Photosynthetic activities

INTRODUCTION

Photosynthesis is of vital importance because of origin of the energy flow in the environment, and cannot be neglected as an eco-physiological parameter for self-sustainable ecosystems. Therefore, the effects of toxins in the environment on photosynthesis should be evaluated. A number of studies have been performed on the responses of the photosynthetic system to heavy metals, ultraviolet radiation, and chemical agents. In the field of radiation protection, protection of the environment from ionizing radiation has recently become an important subject of considerable debate^[1], and it has been also proposed that comparative studies between chemo-ecology and radioecology should be done^[2]. However, few studies have documented the effects of ionizing radiation on photosynthesis. It is important to investigate effects of ionizing radiation on photosynthetic system.

The effects of heavy metals on the photosynthesis of *Anabaena doliolum* and *Chlorella vulgaris* have been investigated at various endpoints, e.g. carbon fixation, O₂ evolution, photosynthetic electron transport and ATP content^[3, 4]. The findings revealed that photosystem

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(PS II) is more sensitive to metals than other components of the photosynthetic system. At present, chlorophyll a fluorescence as an index of PS II has been used to investigate toxic effects on the photosynthetic apparatus, e.g. ultraviolet radiation^[5] and herbicide^[6]. On the other hand, the effects of ionizing radiation on photosynthesis have been reported for only one endpoint in each species, photosynthetic oxygen production of blue-green algae^[7] and sucrose of *Euphorbia-characias*^[8]. It remains unknown whether PS II is most affected by ionizing radiation. It is therefore necessary to investigate the effects on the parameters of photosynthesis in one species.

The aim of this study was to evaluate dose responses of carbon fixation (Specific production rate: SPR), CO₂ utilization efficiency (CUE) and photosynthetic electron transfer rate (ETR) of *Euglena gracilis* Z (*Euglena*) irradiated with gamma-rays up to a dose of 500 Gy. It was also to compare ETR in PS II with other photosynthetic activities (CUE and SPR). Gamma rays of a Cobalt 60 (⁶⁰Co) source, a standard ionizing radiation of relative biological effectiveness (RBE), were used in the present study. *Euglena* has been used to study the physiological chemistry of photosynthesis^[9] and the characteristics of handling for sample adjustment.

MATERIALS AND METHODS

Euglena cells were grown in a half-strength modified #36 Taub and Dollar inorganic nutrient medium^[10] containing ammonium chloride (19.8 mg/L) for autotrophic cultivation. Polycarbonate containers (125 mL) (Nalgene, Rochester, NY) containing 60 mL of the medium were used for *Euglena* culture. All the cultures were incubated at 25°C under a fluorescent lamp of 21 W · m⁻² with a 12-h light-dark cycle.

Ten days after inoculation, solutions containing incubated *Euglena* cells were exposed to five doses of gamma radiation (0, 100, 250, 350 and 500 Gy), at a rate of 71.0 Gy/min using a ⁶⁰Co source. Five containers were irradiated for each dose. The irradiation was carried out at the midterm of an exponential growth phase. For ETR measurements, special conditions were needed, because of the population density required for the detection of chlorophyll a fluorescence. To solve the problem, proteose peptone (Difco Laboratory, Detroit, MI) at 500 mg/L was added to the medium. The irradiation of these samples was also carried out ten days after inoculation. The term was three days into a stationary growth phase, and under pseudo autotrophic conditions.

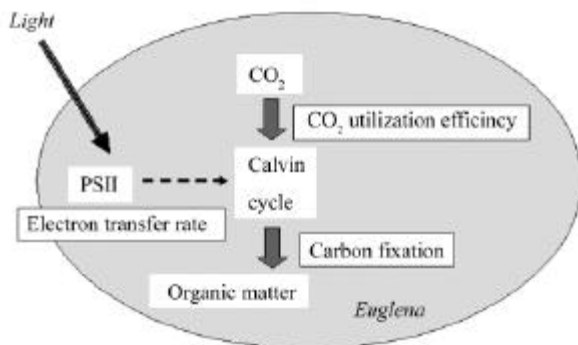


Fig. 1. Outline of three indexes of photosynthetic activity.

Measurements of three indexes (Fig. 1) were carried out 5 days after gamma irradiation,
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because $\delta^{13}\text{C}$ measured for CO_2 utilization efficiency was an average value over a few days^[11].

The ^{13}C tracer methodology was used to evaluate carbon fixation^[12]. A ^{13}C tracer of $\text{NaH}^{13}\text{CO}_3$ with 99 atomic ^{13}C % (Shocomp, Tokyo, Japan) was added to each culture, and the cells were incubated for 12 h under a fluorescent lamp ($21 \text{ W} \cdot \text{m}^{-2}$). A 25-mm diameter glass fiber filter (Whatman GF/F) was heated at 450°C for 4 h. The filtration of *Euglena* cells was carried out at a vacuum pressure of less than 100 mmHg. The filter papers were stored at -20°C until analysis, treated with HCl fumes to remove carbonate, and then completely dried in a vacuum desiccator. The isotopic ratios of ^{13}C and ^{12}C in the samples were determined using a Hitachi RMI-2 mass spectrometer system following the combustion method^[13]. SPR was calculated from isotopic ratios (See details in Appendix A).

CUE was determined by isotope fractionation ($\delta^{13}\text{C}$) (See details in Appendix B). The fractionation was calculated from the model of Takahashi *et al.* (1991) and isotopic ratios. The isotopic ratios of ^{13}C and ^{12}C in the collected samples were determined using the same equipment and methods as for the SPR measurements.

A pulse amplitude modulated fluorometer (Water-PAM, Waltz, Germany) was used to determine ETR^[14]. Chlorophyll a fluorescence analysis was performed in a measuring dark cuvette. ETR was calculated using the formula derived from the fluorescence measurements.

Student's *t*-test at the 5% level ($P=0.05$) was used to determine the difference between irradiated and non-irradiated samples (0 Gy).

RESULTS

Carbon Fixation

The SPR (Table 1) of *Euglena* cells irradiated with 100 and 250 Gy was significantly higher than that of the non-irradiated control, and the SPR of *Euglena* cells irradiated with 500 Gy was decreased as compared with the control (100 Gy, $P = 0.017$; 250 Gy, $P = 0.015$; 500 Gy, $P < 0.0001$ by Student's *t*-test). The carbon fixation of *Euglena* cells was sustained up to 200–300 Gy. Even at a dose of 500 Gy, about half the SPR activity of the non-irradiated control remained.

CO₂ Utilization Efficiency

To determine the CUE, the $\delta^{13}\text{C}$ value was measured, and $\delta^{13}\text{C}$ was estimated assuming that the amount of CO_2 gas in the aqueous phase was -9.0 ‰ ^[11]. **The $\delta^{13}\text{C}$ of *Euglena* cells increased in proportion to the gamma-ray dose (Table 1). The CUE (Table 1) was estimated using Eq. (A2). The CUE values of *Euglena* cells irradiated with 350 and 500 Gy were significantly decreased (350 Gy, $P = 0.022$; 500 Gy, $P = 0.005$ by Student's *t*-test).**

Electron Transfer Rate

The ETR of *Euglena* cells decreased in proportion to the gamma-ray dose (Table 1). A significant decrease was observed at 500 Gy ($P = 0.024$).

DISCUSSION

Chlorophyll a fluorescence as an index of PS II, e.g. ETR, has been used to investigate the effects of metals^[3,4], ultraviolet radiation^[5] and herbicide^[6] on the photosynthetic apparatus. We examined whether PS II was the component of the photosynthetic system most affected

by gamma-ray irradiation. The dose responses of SPR, CUE and ETR were summarized as the relative activity (Fig. 2), which was normalized to the activity of non-irradiated cells.

TABLE 1

Dose Responses of Carbon Fixation, CO₂ Utilization Efficiency and Electron Transfer Rate of Gamma-ray Irradiated *Euglena* Cells. Each Value is the $\bar{x} \pm s$ ($n=5$).

Dose, Gy	Carbon Fixation (SPR), % h ⁻¹	Indexes of Photosynthetic Activity			Electron Transfer Rate (ETR), $\mu\text{mol e} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$
		$\delta^{13}\text{C-PDB}(\%)^b$	$\delta^{13}\text{C}(\%)^c$	CUE, %	
0	0.38 ± 0.048	-32.0 ± 1.2	23.0 ± 1.2	22 ± 4	11.2 ± 0.9
100	0.48 ± 0.048 ^a	-31.2 ± 0.7	22.2 ± 0.7	25 ± 2	11.2 ± 0.5
250	0.53 ± 0.081 ^a	-31.8 ± 0.8	22.8 ± 0.8	23 ± 3	10.8 ± 0.3
350	0.45 ± 0.077	-34.3 ± 1.1	25.3 ± 1.1	14 ± 4 ^a	10.5 ± 0.5
500	0.19 ± 0.015 ^a	-35.1 ± 1.1	26.1 ± 1.1	12 ± 4 ^a	9.0 ± 0.7 ^a

^a Significant differences ($P < 0.05$).

^b The $\delta^{13}\text{C}$ values were estimated with respect to the PDB standard, which is carbonate from the fossil skeleton of *Belemnitella americana* from the Pee Dee formation of South Carolina.

^c Fractionation, $\delta^{13}\text{C}$, was estimated with the assumption that the CO₂ gas in the aqueous phase was - 9.0 ‰¹¹¹.

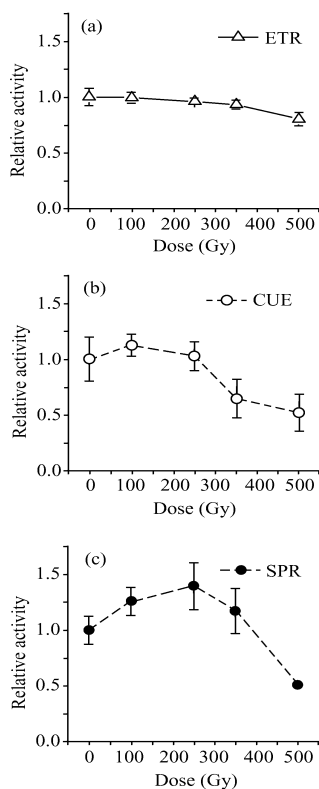


FIG. 2. Relative activity of (a) electron transfer rate (ETR), (b) carbon utilization efficiency (CUE) and (c) specific production rate (SPR) as an index of carbon fixation in the dose range from 0 to 500 Gy. Error bars represent the standard deviation ($n=5$).

The ETR as an index of PS II was sustained at about 80 % of the level in the non-irradiated control, even at a dose of 500 Gy. At 350 and 500 Gy, the relative activity of ETR was higher than that of CUE. This means that the Calvin cycle in *Euglena* was affected by a factor other than radiation injury related to ETR in PS II. In addition, the doses were beyond the lethal dose 50 (LD₅₀, 330 Gy) of *Euglena*^[15]. It was suggested that the enzymatic activity or supply of energy related to the Calvin cycle was markedly altered at a turning point of the LD₅₀. The relative activity of SPR increased up to a dose of 250 Gy. The cause of the increase could be a decrease of carbon consumption due to a suppression of respiration. However, the over-production of sucrose has been reported in *Euphorbia-characias* cells irradiated with gamma-rays at a dose of 250 Gy^[8]. Thus, further study is needed on both the suppression of respiration and accumulation of organic matter.

The radiation dose of 350 and 500 Gy was beyond LD₅₀ of *Euglena* cells. However, it has been reported that even at a 500 Gy dose of gamma-rays, irradiated *Euglena* cells were alive for a very long period (200 days) with no significant difference from the non-irradiated control^[16]. Here, the cells were counted microscopically. The result raises the interesting question whether cells with no proliferation do not alive or have no bioactivity. Our results demonstrate that about half of the photosynthetic activity of non-irradiated cells was maintained in the 500 Gy irradiated cells. The finding suggests that cells under proliferation death may continue the supply of organic material to environment. This is a subject of future research.

In conclusion, our results indicate that the damage to the photosynthetic system caused by gamma-ray irradiation could not be accounted for simply by the effect on PS II. Dose responses of SPR and CUE are very different from those of ETR. Thus, the effects of gamma-ray irradiation on photosynthesis should be evaluated as a whole.

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APPENDIX A SPECIFIC PRODUCTION RATE (SPR) AS AN INDEX OF CARBON FIXATION

SPR is the carbon uptake rate per unit carbon mass. The SPR ($\hat{g} \text{ C} / \hat{g} \text{ C} \cdot \text{h}^{-1}$) is defined as follows:

$$\text{SPR} = \frac{(a_{is} - a_{ms})}{(a_{ic} - a_{ms})} \times \frac{1}{\Delta t} \quad (\text{A-1})$$

where, a_{ns} and a_{ic} are ¹³C atomic % in *Euglena* and the medium at the start point of the ¹³C tracer experiment, respectively. a_{is} is the ¹³C atomic % of *Euglena* at the end of the ¹³C tracer experiment. Δt is the incubation time of the ¹³C experiment. The SPR is zero until *Euglena* cells assimilate the tracer of ¹³C by photosynthesis and partly decreases with respiration after the uptake of ¹³C.

APPENDIX B ISOTOPE FRACTIONATION ($\delta^{13}\text{C}$) AND CO_2 UTILIZATION EFFICIENCY

The $\delta^{13}\text{C}$ value can be related to isotope fractionation ($\Delta^{13}\text{C}$) by:

$$\Delta^{13}\text{C} (\%) = \delta^{13}\text{C}(\text{CO}_2 \text{ aqueous phase}) - \delta^{13}\text{C}(\text{Euglena}), \quad (\text{B-1})$$

where $\Delta^{13}\text{C}$ is estimated from the measured $\delta^{13}\text{C}(\text{Euglena})$ and $\delta^{13}\text{C}$ (CO_2 aqueous phase) values of dissolved CO_2 ($-9 \text{‰}^{[11]}$). The basic relation between $\Delta^{13}\text{C}$ and CUE is given by:

$$\Delta^{13}\text{C} = b + (a - b) \times \text{CUE}, \quad (\text{B-2})$$

where a and b are fractionation factors associated with the processes of CO_2 diffusion through the membrane, $0.7 \text{‰}^{[17]}$ and carboxylation by Rubisco (ribulose biphosphate carboxylase-oxygenase), $29.4 \text{‰}^{[18]}$, respectively.

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