Effect of Gamma Irradiation on Uracil-^{14}C Incorporation into *Euglena* Cells

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Radiosensitivity of various kinds of RNA in *Euglena* cells of two different growth phases, logarithmic and stationary, of culture to 1×10^5r gamma irradiation was investigated by labelling these RNAs with uracil-^{14}C and fractionating them chromatographically. The raw nucleic acid of the logarithmic phase cells decreased more in quantity than that of the stationary phase cells after the irradiation. When this raw nucleic acid was fractionated by MAK** column chromatography, all the fractions obtained decreased in u. v. absorbancy in the case of irradiated cells as compared with the case of non-irradiated cells. The decrease rate in u. v. absorbancy of all the fractions became low in the order of eluting sequence, A, B, C and D in the irradiated logarithmic phase cells, and in the order of B, A, C and D in the irradiated stationary phase cells. Although the RNA level was affected by irradiation in large quantity, a slight uracil-^{14}C incorporation into RNA was recognized in the irradiated logarithmic phase cells, while in spite of a little change in the RNA level, uracil-^{14}C incorporation into RNA was hardly recognized in the irradiated stationary phase cells.

INTRODUCTION

It has heen reported that an irradiation of *Escherichia coli* with small doses of ultraviolet irradiation causes a temporary cessation of DNA synthesis without markedly affecting the overall synthesis of RNA and protein.[1,2] But, a little is known as to similar irradiation effects on algal cells. According to the current literature,[3,4,5] *Euglena* cells contain, along with sRNA and rRNA, a DNA-like RNA which may be designated as mRNA. The present author has reported that the RNA level of logarithmic phase cells is more unstable to gamma irradiation than that of stationary phase cells in *Euglena*.[6] It seems desirable to know whether these different kinds of RNA show different sensitivity to gamma irradiation or not. In recent reports, Wainfan *et al.*[7] have indicated that an irradiation dose which partially inhibited rRNA synthesis does not affect sRNA synthesis. Moreover, Sibatani and Mizuno[8] have reported that the RNA synthesis is impaired progressively with increasing doses of ultraviolet irradiation. They have also reported that the oder of u. v. sensitivity was that of increasing molecular weight of sRNA and rRNA. The present paper deals with the effect of gamma irradiation on the uracil-^{14}C incorporation into different kinds of RNA in *Euglena* cells in two

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** Abbreviations: MAK; methylated serum albumin-kieselguhr, RNA; ribonucleic acid, sRNA; soluble RNA, rRNA; ribosomal RNA, mRNA; messenger RNA.

different growth phases, logarithmic and stationary.

MATERIAL AND METHODS

Material. Logarithmic and stationary phase cells of *Euglena gracilis var. bacillaris*, Camb. Coll. No. 1224/7, were used in the present work. About 4×10^6 stock cells were incubated for 5 days in 1500 ml of fresh culture medium[9] under the culture conditions given in the following description. These incubated cells were used as "logarithmic phase cells" in the present work. Similar stock cells incubated for 20 days in the same manner as above were used as "stationary phase cells."

Irradiation. The cells were washed centrifugally with distilled water. By this washing, dead cells were eliminated from the living ones. Only the latter cells were resuspended in 200 ml of distilled water. One half of this cell suspension was exposed to 1×10^5r gamma rays and the other half was used as the control. The irradiation was carried out with the ^{60}Co gamma ray irradiation facility of the Institute for Chmical Research of Kyoto University. The dose rate of this facility was 1.04×10^5 r/hr.

Culture after Irradiation. Each of 100 ml aliquot of the non-irradiated and the irradiated cell suspensions was transferred into 1400 ml of fresh culture medium, which was of one tenth strength of the usual culture medium. Then all the cell suspensions were incubated under illumination (about 2500 lux) for 12 hours at 30°C without shaking and aeration. No effect of dilution of culture medium was recognized on cell growth at least during the fresh culture medium, 50μc of uracil-^{14}C was dissolved into the same culture medium. Accordingly, uracil-^{14}C concentration was about 33 $\mu\mu$c/ml in the beginning of culture, this uracil-^{14}C concentration did not affect the cell growth. The uracil-^{14}C used obtained from the Radiochemical Centre at Amersham through the Daiichi Kagaku Co. in Japan.

Preparation and Analysis for Nucleic Acids. All the non-irradiated and irradiated cells incubated after irradiation were washed thoroughly with distilled water. The washed cells were disrupted with a Kubota KMS-100 sonic vibrator at 3°C for 3 minutes in 30 ml of 0.1 M buffered saline (0.1 M sodium chloride in 5 mM phosphate buffer, pH 6.7) containing 5 mM magnesium sulfate and 0.2% laulylsulfate. After the cell disruption, nucleic acids were extracted from the homogenate obtained according to the procedure of Kirby.[10] The raw nucleic acid obtained was absorbed to MAK column,[11,19] and eluted with increasing concentration of sodium chloride (0.1 to 1.0M) according to the procedure of Mandell and Hershey.[11] The effluents were put into 120 tubes, each containing 5 ml, according to eluting sequence. The effluents were examined for absorbancy at 260 mμ with a Hitachi EPU-2 spectrophotometer. The relative amount of total nucleic acids in a given fraction was expressed by the total absorbancy at 260 mμ. The presence of DNA and RNA in the effluents was examined by color reactions with indole,[12] diphenilamine,[13] and orcine[14] reagents respectively. The sodium chloride concentration in the effluents was determined by Mohr's method.[15] The radioactivity of uracil-^{14}C was measured with a Kobe-Kogyo PC-26 gas-flow counter.

RESULTS

1) Absorption Spectra of Raw Nucleic Acid

The raw nucleic acid obtained from phenol extract was dissolved into 30 ml of 0.1 M buffered saline of pH 6.7. The abrorption spectra of these raw nucleic acid solutions were shown in Fig. 1, A and B. In both non-irradiated and irradiated logarithmic phase cells, these absorption spectra showed a maximum at about 258 mμ, and a minimum at about 232 mμ. But the amount of the raw nucleic acid of the irradiated cells decreased by about 29.1 % as compared with that of the non-irradiated cells (Fig. 1, A ; -○-, -●-). In the stationary phase cells, the absorption spectrum of the raw nucleic acid of the non-irradiated cells showed a maximum at 261 mμ and minimum at 237 mμ, and that of the irradiated cells a maximum at 258 mμ and a minimum at 235 mμ (Fig. 1, B ; -○-, -●-). After the irradiation, the amount of the raw nucleic acid of the irradiated cells decreased by about 15.1 % as compared with that of the non-irradiated cells.

2) Identification of Fractions Obtained by MAK Column Chromatography

When the raw nucleic acid of the non-irradiated logarithmic phase cells absorbed on MAK column was eluted with increasing concentration of sodium chloride, four fractions were obtained (Fig. 2). The chromatographic pattern obtained was similar to those of *E. coli*,[19] HeLa cells,[17] and *Psudomonas*-p.[18] For the convenience's sake, all the main fractions obtained were designated as A, B, C and D. Besides these four fractions, a fraction containing the substance which passed freely through MAK column was obtained (Fraction R), when the raw nucleic

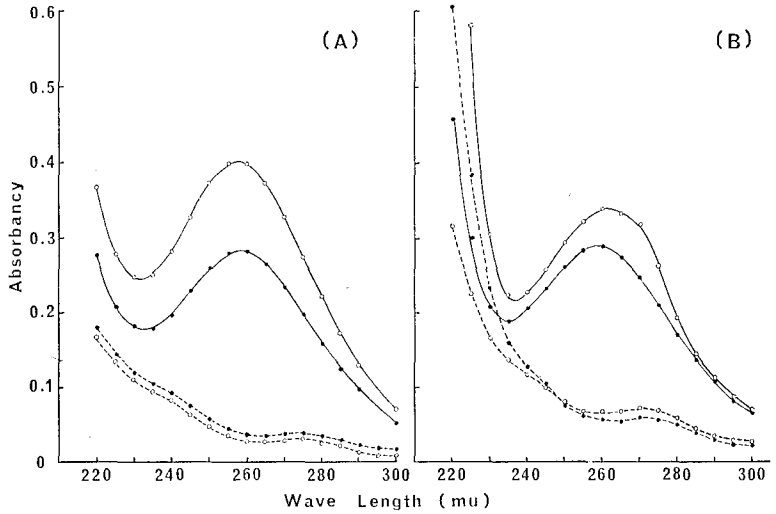

Fig. 1. Absorption spectra of raw nucleic acid and runthrough substances obtained from non-irradiated and irradiated cells.
(A) ; logarithmic phase cells, (B) ; stationary phase cells. Non-irradiated cells ; (-○-), irradiated cells ; (●). Runthrough substances of non-irradiated cells ; (···○···), those of irradiated cells ; (···●···). The cells used for extraction were about 1×10^8 in number.

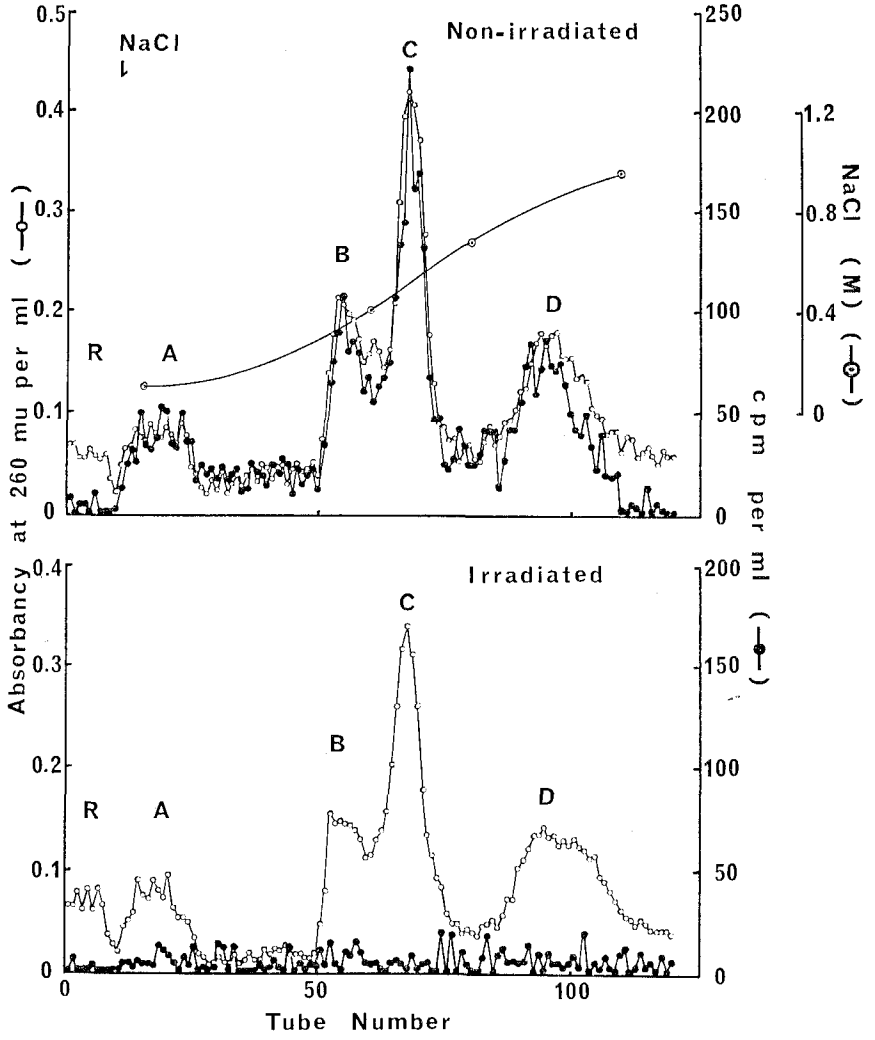

Fig. 2. A chromatographic pattern of nucleic acids of the non-irradiated and the irradiated logarithmic phase cells fractionated by MAK column chromatography.
The cells used for extraction were about 3×10^8 in number. The effluent volume of every tube was 5 ml. Cpm; count per minute of uracil-^{14}C. Column; 1.5×20 cm. Flow rate; 30 ml/hr.

acid was added to the column. This runthrough substance showed tow small absorption maxima at about 240 and 275 mμ (Fig. 1, A; ···○···, ···●···). On the other hand, the runthrough substance from the irradiated stationary phase cells was optically denser in the ultraviolet range from 220 to 240 mμ than that from the non-irradiated cells (Fig. 1, B; ···○···, ···●···). The fraction A eluted with about 0.1 M sodium chloride was positive in all three color reactions mentioned above. The absorption spectrum of the effluent showed a maximum at about 270 mμ and a minimum at 237 mμ (Fig. 4). This fraction is identified as that of nucleotides by many investigatiors.[16,17,18] The fraction B eluted with about 0.3 M sodium chloride was positive in orcinol color reaction, and negative in indole and dipheni-

lamine color reactions. The absorption spectrum of this fraction showed a maximum at about 257 mμ. Accordingly, this fraction seems to contain RNA, but does not contain DNA. The RNA of this fraction is well known as sRNA. The fraction C eluted with about 0.5 to 0.6 M sodium chloride seems to contain a large amount of DNA and a small amount of RNA, because the effluent of this fraction was apparently positive in indole and diphenilamine reactions, and slightly positive in orcinol reaction. The absorption spectrum of this fraction showed a maximum at about 258 mμ. Though this fraction is generally known as that of DNA, the present fraction was a mixture of DNA and RNA. The fraction D eluted with about 0.8 M sodium chloride seems to contain RNA, because they were apparently positive in orcinol reaction, and negative in indole and diphenilamine reactions. The aborption spectrum of this fraction showed a maximum at about 258 mμ. The RNA of this fraction is well known as rRNA. These chemical and spectral characteristics of all the fractions mentioned above were reproducible in the nucleic acid samples from both non-irradiated and irradiated cells. Accordingly, when a given fraction showed the same color reaction and ultraviolet absorption spectra as described above, the fraction was designated as A, B, C and D just as in the case of the non-irradiated logarithmic phase cells.

3) Amount of U. V. Absorbing Substances in Each Fraction

The relative concentration of nucleotides or nucleic acids in each fraction obtained from the non-irradiated and irradiated cells of both growth phases was estimated by total absorbancy at 260 mμ and is shown in Table 1. The fraction A of the irradiated logarithmic phase cells decreased in quantity by about 38 % as compared with that of the non-irradiated cells, the fraction B by about 18 % etc.. The decrease rate in u. v. absorbancy of every fraction became low in the order of eluting sequence from MAK column ; A, B, C and D. Thus, the total amount of the u. v. absorbing substances of the irradiated logarithmic phase cells decreased by about 18 % as compared with that of the non-irradiated cells.

Table 1. Decrease rate of u. v. absorbing substances after irradiation.

Fraction	A	B	C	D	Total
Log. phase cell	38.4±1.6	18.3±2.1	16.5±4.8	5.0±2.4	18.4±0.4
St. phase cell	8.3±2.5	9.6±1.2	4.9±1.6	3.2±1.2	5.1±0.6

Total ; total of all the effluents containing u. v. absorbing substances. All the values in percentage are the averages of the results of three experiments.

The factions from the irradiated stationary phase cells also decreased in quantity. But the decrease rate of each fraction was generally less than that from the irradiated logarithmic phase cells. That is, the fraction A decreased by about 8 % as compared with that of the non-irradiated cells, the fraction B by about 10 % etc. In this case, the decrease rate in u. v. absorbancy of every fraction became low in the order of B, A, C and D. The total amount of the u. v. absorbing substances of the irradiated cells decreased by about 5 % as compared with that of the non-irradiated cells.

By this reason, it may be concluded that sRNA was more unstable to gamma-irradiation than rRNA in both growth phase cells.

4) Incorporation of Uracil-^{14}C into Nucleic Acids

When the non-irradiated logarithmic phase cells were incubated for 12 hours in the fresh culture medium containing uracil-^{14}C, the uracil-^{14}C was incorporated in large quantity into the incubated cells (Fig. 2 ; -●-). When the raw nucleic acids were extracted with phenol from the non-irradiated and the irradiated cells in two growth phases, and fractionated by MAK column chromatography as described above, uracil-^{14}C incorporation was recognized in large quantity in the fractions of A, B, C and D, though only a slight incorporation was found in fraction R. The degree of uracil-^{14}C incorporation was in parallel with the u. v. absorbancy of each fraction. As the fraction C contains a small amount of RNA together with DNA, this fact may partly account for the high uracil-^{14}C incorpora-

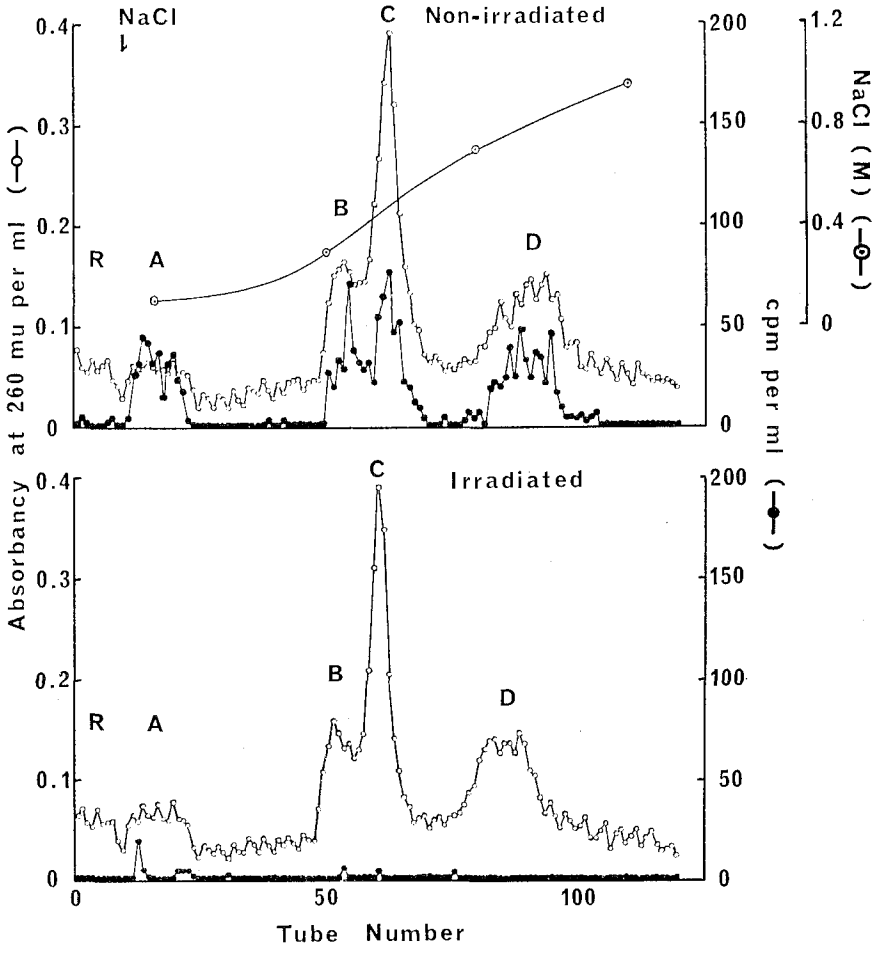

Fig. 3. A chromatographic pattern of nucleic acids of the non-irradiated and the irradiated stationary phase cells fractionated by MAK column chromatography.
The cell number and all the analytical methods were same as those described in Fig. 2.

tion into this fraction. With regard to this high incorporation, a detailed analysis will be required (cf. the report of Comings[20]). Moreover, in this experiment, uracil-^{14}C incorporation was also recognized to a considerable extent into endogenous substances. Contrary to this, when the irradiated logarithmic phase cells were incubated and analysed in the same manner as above, only a slight uracil-^{14}C incorporation into the cells was recognized (Fig. 2). From these results, it is concluded that the RNA synthesis is carried out to reduced extent in the irradiated logarithmic phase cells.

When the non-irradiated stationary phase cells were incubated with uracil-^{14}C and were analysed in the same manner as above, the incorporation rate of uracil-^{14}C into these cells was considerablly small in comparison with the case of the non-irradiated logarithmic phase cells (Fig. 3 ; -●-). Especially, the incorporation was hardly recognizable into endogenous substances of these cells. Contrary to this, when the irradiated stationary phase cells were examined as to the uracil-^{14}C incorporation in the same manner as above, uracil-^{14}C incorporation into these cells was hardly recognizable. Therefore, it may be concluded that the RNA synthesis in the irradiated stationary phase cells is almost perfectly inhibited by irradiation.

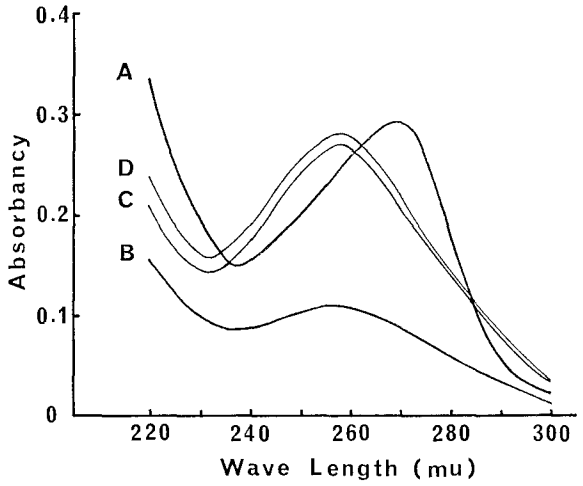

Fig. 4. Absorption spectra of nucleic acids fractionated by MAK column chromatography.

DISCUSSION AND CONCLUSION

Many investigations have recently been carried out with regard to the nucleic acids of *Euglena* cells.[3-9,19] For instance, Blum and Buetow[19] have found that the addition of actinomycin D to logarithmically growing *Euglena* cells results in an inhibited growth and RNA synthesis of these cells. But only a little is known as to the effect of ionizing irradiation on growth and nucleic acid synthesis of *Euglena* cells. Sibatani and Mizuno[8] have reported that the RNA synthesis of *E. coil* irradiated with ultraviolet light is impaired progressively with increasing doses. They have also found that the order of sensitivity to irradiation is in parallel with the in-

creasing molucular weight of RNAs, such as transfer RNA and ribosomal RNA. But the results obtained in the present study rather contradict the above mentioned view. That is, the degree of breakage of the RNAs of logarithmic phase cells of *Euglena* was small according to the order of increasing molecular weight. Such a difference in radiosensitivity of RNAs may be due to the difference in material used, radiation used and in radiation dose.

It has previously been reported[9] that the RNA level of the logarithmic phase cells is more unstable to gamma irradiation than that of the stationary phase cells. An evidence which supports this view was also obtained in the present work. The amount of all the u. v. absorbing substances eluted from MAK column decreased by about 18% in the case of irradiated logarithmic phase cells and by about 5% in the case of irradiated stationary phase cells. These results are shown in Table 1 in detail. From this table, it may be concluded that in the logarithmic phase cells both intracellular nucleotides and sRNA play an important role in the change of nucleic acid level after irradiation, judging from the high irradiation damage of these two fractions, A and B. Such a characteristic feature was also found in the case of the stationary phase cells to a less extent.

It has also been reported[9] previously that the logarithmic phase *Euglena* cells were more resistant to gamma irradiation than the stationary phase cells on the basis of the following two reasons : first, the irradiated logarithmic phase cells showed more rapid growth recovery than the irradiated stationary phase cells. second, the former cells were rather small in death rate than that of the latter ones. An evidence which may suport the above mentioned view was obtained in the present study, that is, the uracil-^{14}C incorporation into RNA was slightly recognized in the irradiated logarithmic phase cells, but hardly recognizable in the irradiated stationary phase cells. Accordingly, it may be reasonable to conclude that although the RNA metabolism of the irradiated logarithmic phase cells is largely inhibited as compared with that of non-irradiated cells, a slight metabolic activity was recognizable in these cells, while any activity was hardly recognizable in the irradiated stationary phase cells.

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