Bull. Inst. Chem. Res., Kyoto Univ., Vol. 46, No. 1, March 1968

An Effect of Gamma Irradiation on Solution of Soluble and Ribosomal RNAs

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Received December 28, 1967

From growing *Euglena* cells, RNA** was extracted by phenol method and separated into s- and r-RNAs with MAK column. Both RNA solutions (RNA solved in 0.1 M phosphate buffered saline, pH 6.7) were irradiated with Co-60 gamma rays in doses of 1× and 2×10^5 R, and then examined spectrophotometrically. Irradiation of these solutions gave a proportional increase in absorption to dose. And the irradiadiated s-RNA showed slightly a red shift in λ_{max} with dose. When these irradiated solutions were heated at different temperatures, they changed as follows: (1) The decrease of optical density of s-RNA was inversely proportional with dose at temperatures of 40°to 80°C. (2) λ_{max} of s-RNA showed a slight blue shift with dose. (3) Irradiation of r-RNA gave no shift in λ_{max}, but the optical density at 260 mμ incresed linearly with dose. (4) Absorption of irradiated s-RNA solution increased sigmoidally with increasing amount of heat, while that of the irradiated r-RNA solution increased almost linearly with heat. (5) Tm of both irradiated RNAs was slightly higher than that of the non-irradiated RNAs. (6) s-RNA was slightly more sensitive to gamma irradiation than r-RNA.

INTRODUCTION

It is well known that some physical effects, such as heat, irradiation, cause a change of molecular structure of nucleic acid. For instance, the absorption of ultraviolet light by nucleic acid solution increases with increasing amount of heat[1,2], and the ionizing irradiation of undenaturated DNA solution is known to give a similar effect[3,4]. These have been attributed to loss of hydrogen bonding between bases of double-strand structure of nucleic acid, by heat or by irradiation. However, the mechanism of increase of absorption by heat may differ from that by irradiation. The irradiation effect on nucleic acid contains more irreversible factors than the thermal effect. That is, the heat is known to dissociate the molecule of nucleic acid reversibly, and to denaturate it without loss of components to a considerable extent, whereas the irradiation disrupts irreversibly some of components of the molecule with a dissociation of certain chemical bondings.

The effect of irradiation or heat may be different with the kind of RNA. Further, it may vary between RNA *in vivo*[5,6] and RNA *in vitro* in the same RNA. The present work has been undertaken in order to obtain some information concerning these problems.

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** Abbreviations: s-RNA, soluble ribonucleic acid; r-RNA, ribosomal RNA; DNA, deoxyribonucleic acid; MAK, methylated serum albumin kieselguhr; λ_{max}, wave length of absorption maximum; Tm, mean temperature of thermal unfolding.

The present study deals with the following four points : (1) Irradiation damage of RNA *in vitro,* (2) irradiation dose which is affectable to RNA *in vivo,* (3) change of irradiation effect by heat, and (4) difference of irradiation effect between s- and r-RNAs.

MATERIAL AND METHOD

Materials used were s- and r-RNAs of growing cells of *Euglena gracilis var. bacillaris.* The extraction of RNA was carried out by Kirby's phenol method[7], and then its separation was made according to the method of MAK column[8]. Thus, both RNAs separated were dialysed in 0.1 M phosphate beffered saline for 10 hours at 5°C. The dialysed RNA solution (60 ml) was separated equally in volume into three eliquots, two for gamma irradiation of $1\times$ and 2×10^5 R and the rest for control. Immediately after irradiation, 37 % formalin[9] of 0.5 ml was added to each solution in order to prevent the recombination of free bases produced by irradiation. The irradiation was carried out with a Co-60 irradiation facility of the Institute for Chemical Research of Kyoto University. The dose rate of this apparatus was 0.92×10^5 R per hour. The temperature of RNA solution was about 10°C during irradiation. After the irradiation, all the non-irradiated and irradiated solutions were examined spectrophotometrically, first at room temperature (16°C) and then with increasing amount of heat, by means of heating in a water bath for 20 min. at several level of temperature range from 20 to 80°C.

RESULTS

1) Change of absorption spectra at room temperature

After the irradiated s-RNA solution was examined at room temperature (16°C), and it was found that irradiation gives a slight increase in absorption of ultraviolet

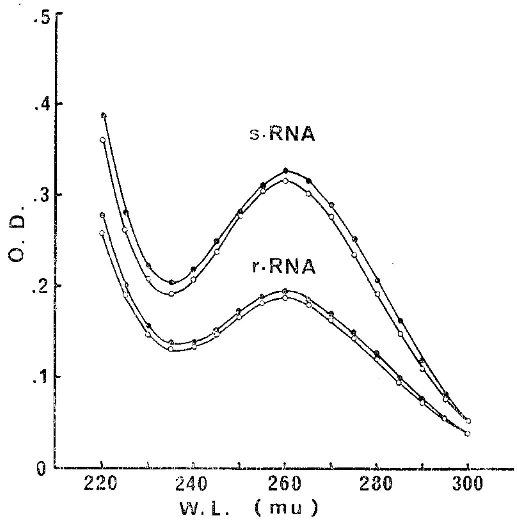

Fig. 1. Absorption spectra of non-irradiated and irradiated RNAs at 16°C. Non-irradiated (-○-), 2×10^5 r irradiated (-●-)

Table 1. Change of absorption of s- and r-RNAs by gamma irradiation and by heating.

Treatment		s-RNA		r-RNA	
Irradiation (r)	Temperature (°C)	λ_{max} (mμ)	O. D. increased at 260 mμ (×)	λ_{max} (mμ)	O. D. increased at 260 mμ (%)
Non-irradiated	16	259	0	260	0
1×10^5	16	260	0.9	260	1.6
2×10^5	16	261	2.5	260	2.6
Non-irradiated	80	263	25.1	262	24.7
1×10^5	80	262.5, 263	24.5	262	27.4
2×10^5	80	262, 262.5	23.2	262	30.0

light, and the rate of the increase was proportional to irradiation dose (Fig. 1 and Table 1). Further a slight red shift was observed in λ_{max} of the irradiated s-RNA. On the other hand, a similar increase of absorption was also observed in the irradiated r-RNA, however, no shift of λ_{max} was recognized in this case (Table 1, upper part). The degree of increase of absorption of both s- and r-RNA solutions irradiated with 2×10^5 R almost corresponds to those of the their non-irradiated solutions heated at about 35°C for 20 min.

2) **Change of irradiation effect by heating**

It is well known that when RNA solution is heated, the ultraviolet absorption of the RNA solution increases sigmoidally with increasing amount of heat, especially within the temperature range of from 40 to 80°C. This was confirmed in the present study, as shown in Fig. 2. That is, the optical density at 260 mμ rapidly increases both in the non-irraiated and the irradiated s-RNA solutions over the range from 35 to 60°C. But the data of the irradiated solution differed from that of the non-irradiated solution in the following point; the former was higher than the latter in the region from 16 to 40°C, and lower in the region from 40 to 80°C (Fig. 2, upper part). The optical density at 80°C is higher by about 25% than that at 16°C in the non-irradiated s-RNA solution, and higher by about 20% in the 2×10^5 r irradiated case (Table 1). This difference (about 5%) is obviously due to the presence and absence of the radiation effect, indicating a decrease of allowableness of thermal unfolding on the base composition of s-RNA. This fact also suggests that some of base compositions of s-RNA are disrupted by gamma irradiation.

Tm of the s-RNA used was about 47°C in the non-irradiated solution and about 50°C in the irradiated solution. The former value (47°C) was slightly lower than that reported by Spirin[10] on TMV-RNA (49°C) or *E. coli* RNA (54.5°C). It was reported by Marmur and Doty[1] that Tm of a nucleic acid depends on the guanine and cytosine content of the nucleic acid. Therefore, in the present study, the difference of Tm between non-irradiated and irradiated s-RNAs may be attributed to some losses of adenine and uracil content by irradiation.

The s-RNA solution heated at 80°C showed an increase in absorption with a slight red shift of λ_{max}. But the degree of increase was reduced proportionally to the irradiation doses. Further, a similar decrease was also observed in λ_{max}

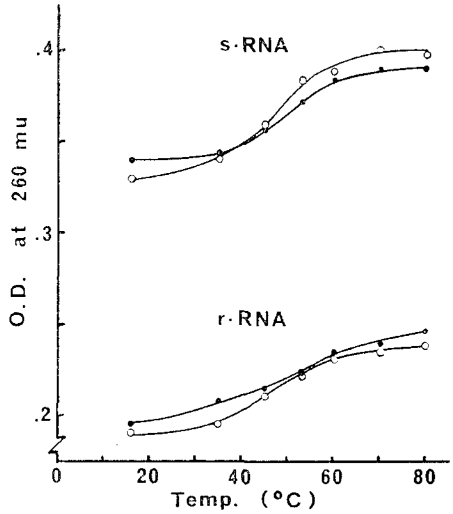

Fig. 2. Change of absorbancy of non-irradiated and irradiated RNAs with increasing amount of heat.
Non-irradiated (-○-), 2×10^5 r irradiated (-●-)

(Table 1, lower part).

On the other hand, the absorption of the non-irradiated r-RNA was increased sigmoidally with elevation of temperature of solution, while in the irradiated r-RNA it was increased linearly with temperature under the same heat treatment as mentioned above. And the optical density of irradiated r-RNA was always higher than that of non-irradiated one over the region from 16° to 80°C. These suggest that the r-RNA had lost parts of chemical bondings after irradiation, and was changed into a subpolymer structure uniformalized for thermal effect, without loss of any component by destruction of irradiation.

Tm of the r-RNA used was about 48°C in the non-irradiated solution, and about 50°C in the 2×10^5 R irradiated solution. When the non-irradiated r-RNA solution was heated at 80°C, the optical density at 260 mμ of this solution increased by about 25% as compared with that at 16°C. This increase rate of optical density was almost same as that of s-RNA mentioned above. Further, a slight red shift of λ_{max} was also observed in this case. When the irradiated r-RNA solutions were heated at the same temperature, the optical density of these solutions increased proportionally to irradiation dose (Table 1, lower part). But no change was observed in λ_{max} of these solutions.

DISCUSSION

Gamma irradiation of 1×10^5 R level gives a considerable effect on the synthesis of *Euglena* RNA *in vivo*[5,6]. This irradiation level seems to be insufficient to give a large effect on the RNA *in vitro*, such as destruction of molecule, but it brings about a slight change on the RNA molecule. The double strand structure of nucleic acid is kept intact in relatively low temperature. And heating of nucleic

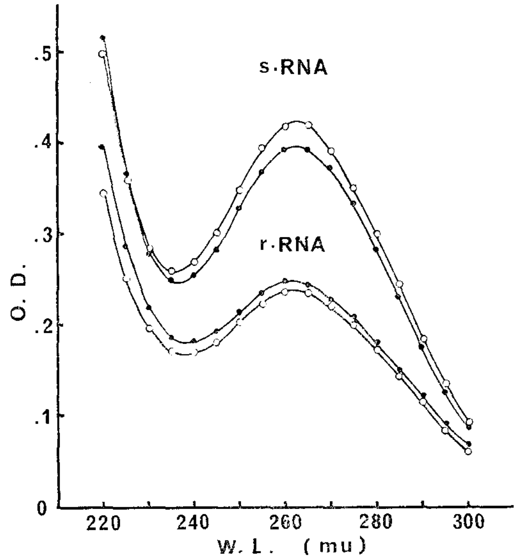

Fig. 3. Absorption spectra of non-irradiated and irradiated RNAs after heating their solutions at 80°C.
Non-irradiated (-○-), 2×10^5 r irradiated (-●-)

acid solution gives an increase in absorption with increasing amount of heat. This has been attributed to a loss of hydrogen bonding of their structure. The fact that gamma irradiation of RNA solution leads to a similar increase in absorption may suggest the liberation of some basic substances after irradiation, in addition to loss of hydrogen bonding at least in the RNA solution below 35°C. These irradiation effects of RNA were expanded in the heated solution. This seems to be attributed to activation of free radicals produced by irradiation with the temperature over about 40°C. Therefore, by reaction of the free radicals liberated by irradiation and activated by heat, a RNA molecule, which was kept in the state of a slight damage at low temperature, may disintegrate into some fragments[11]. Consequently, the decrease in molecular weight of the irradiated RNA becomes distinctly after heat treatment of the solution.

There is a well known fact that the mean temperature (Tm) of thermal unfolding of nucleic acid structure increases linearly with guanine and cytosine content in this acid. In the present study, it was also found that Tm of irradiated RNA was slightly higher than that of non-irradiated RNA. This suggests that the irradiated RNA is relatively rich in guanine and cytosine content than the non-irradiated RNA. This suggestion was recently confirmed by analysis of base composition[12]. Furthermore, there are some data that uracils are easily broken by gamma irradiation incomparison with other bases of nucleic acid[13,14]. Accordingly this also may become a help to loss of adenine and uracil of RNA by irradiation.

The author wishes to express his appreciation to Dr. Y. Tamura for encouraging the performance of this study and Dr. M. Fujimoto for his valuable advice in writing this paper. And his cordial thanks are due to Prof. S. Shimizu for giving the chance to use the gamma-ray irradiation facility of his institute. He is also indebted to Mr. R. Katano for his kind cooperation in the irradiation procedure.

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