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Radiation Damage of Purines and Pyrimidines

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Purine and pyrimidine bases and their nucleotides with the different number of phosphoric acid residues were seperated from the aqueous solution of ATP, GTP, CTP and UTP with Dowex-1-formate column. Utilizing this preparation, the above four triphosphate nucleotide solutions were irradiated with 2×10^5 R gamma rays and then the degree of their irradiation damage was investigated. With respect to the radiosensitivity, it was found that the compounds were resistant to gamma irradiation in the order of adenine, guanine, cytosine and uracil compounds. This may be due to the number of hydroxyl groups of the molecule of compounds studied. The fact that purines were more radioresistant than pyrimidines may be due to the difference of molecular weight. Futher, in purine nucleotides the radioresistancy was proportional to the number of their phosphoric acid residues.

INTRODUCTION

On the way of the nucleotide investigation of gamma irradiated *Euglena* cells, it was frequently noticed that the extract of the irradiated cells contained more quantity of the purine nucleotides than the pyrimidine nucleotides[1,2]. This suggests that the nucleotides with different base components may have its own radiosensitivity.

Several data have been reported by some investigators on the effect of ionizing irradiation on purines and pyrimidines[3~8]. Guzman *et al.*[6] have reported on x-irradiation study of purines and pyrimidines that the molecule became more resistant to the ionizing radiation, as it became more complex on addition of other groups, such as pentose and phosphoric acid residues. Though similar result was recognized in the present study within the same base compounds, this principle was not necessarily to be the case among different bases. But in order to clear this problem, some more interpretable data were needed for a systematic study of radiation effect on these compounds. The present study deals with the comparison of effect of radiation among the four different base compounds.

MATERIALS AND METHODS

Materials used in the present study were adenosine-triphosphate (ATP), guanosine-5′-triphosphate (GTP), cytidine-5′-triphosphate (CTP) and uridine-5′-triphosphate (UTP). ATP was purchased from Daiichi Kagaku, Tokyo and the other three compounds from Sigma Chemical Co., Missouri, USA.

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One mg of each compound was dissolved in 10 ml of distilled water, respectively. A half of this 10 ml aqueous solution was irradiated with a dose of 2×10^5 R in the Co-60 gamma irradiation facility of Institute for Chemical Research of Kyoto University. The dose rate of the facility was 1.33×10^5 R per hour. The other half was used as the control.

After the irradiation, all the irradiated and non-irrdiated compounds were absorbed to Dowex-1-formate column (200~400 mesh) prepared, respectively, and then eluted with a slightly modified eluting solution of Cohn and Volkin[9,11]. Five ml elutes were collected with constant flow of about 8 drops per min at 10°C. The absorbancy of elutes was read at 260 and 275 mμ, respectively. The identification of the fraction obtained was made by means of absorption spectra and phosphorus determination[10]. The relative amount of effluent in a given fraction was determined by total absorbancy at 260 mμ of the fraction. The correction for the background absorbancy of the eluting solution was made by deducting the absorbancy of eluting solutions from that of the elute.

RESULTS

1) Purines

When non-irradiated ATP and GTP were separated with Dowex-1-formate column, respectively, at least 5 fractions were obtained in each case. For convenience these fractions were designated as B, M, D, T and U according to the sequence of elution from the column (Figs. 1 and 2). Fraction B contained the base component of each case, such as adenine in ATP and guanine in GTP.

Tabble 1. Relative quantity of the non-irradiated purine and pyrimidine compounds fractionated with Dowex-1-formate column.

Substance	Fraction					
	B	M	D	T	U	Total
ATP	4.5	24.1	33.3	31.3	6.8	100
GTP	7.6	25.2	43.3	20.8	3.1	100
CTP	7.0	22.6	34.0	27.5	8.9	100
UTP	5.4	19.6	36.7	33.3	5.0	100

B : base, M : monophosphate, D : diphosphate, T : triphosphate, U : unidentified substance

Table 2. Comparative effect of gamma irradiation on purine and pyrimidine compounds

Substance	Fraction				
	B (%)	M (%)	D (%)	T (%)	U (%)
ATP	342.5*	50.2	39.5	32.9	14.8
GTP	79.0	80.9	78.7	45.7	46.9
CTP	41.1*	70.5	83.9	86.3	17.7*
UTP	33.7	79.9	91.1	78.1	52.6

* : increase rate, the rest : decrease rate

Fraction M contained the monophosphate, fraction D the diphosphate, and fraction T the triphosphate. The substance of fraction U was not identified because of a small quantity in the present work. The fractions obtained from non-irradiated ATP had an absorption maximum at about 259 mμ. The relative quantity obtained is shown in Table 1.

When such a ATP solution was irradiated by gamma rays, and then investigated in the same manner as stated above, the fractions obtained remained unchanged in number, sequence of elution, and absorption spectra. Fraction B increased in quantity by about 4.4 fold after the irradiation, but the remaining 4 factions straightly decreased in quantity according to eluting sequence (Table 2). That is, among fractions M, D and T, as the phosphoric acid increases in number, the nucleotide became resistant proportionally to the action of gamma irradiation. The increase of fraction B dependeds principally on the release of free adenine and on the high resistancy of adenine to gamma irradiation. Fraction U seems to suggest that it may contain adenosine-tetraphosphate[12], because the decrease rate of the fraction diagrammatically corresponds to that of the substance.

The result of GTP obtained in the same manner as in the case of ATP

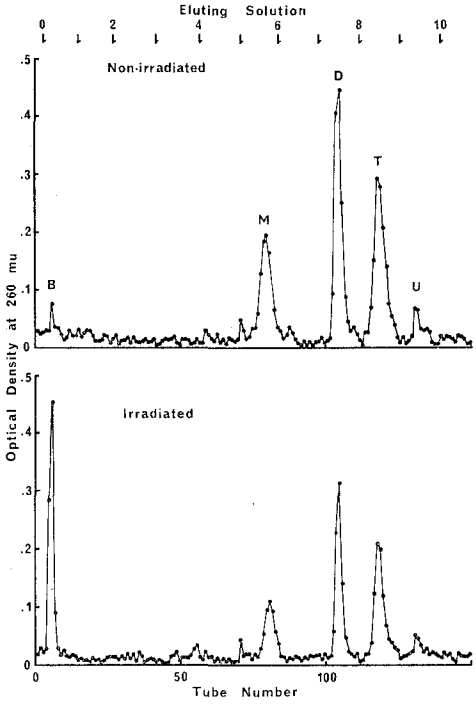

Fig. 1. Chromatographic patterns of the adenine compounds obtained from ATP material.
Column : Dowex-1-formate, 1×12cm
Eluting solution : (1) 0.01M ammonium formate (AF), (2) 0.02M AF, (3) 0.15N formic acid (F), (4) 0.05M AF+0.01N F, (5) 0.1M AF+0.1N F, (6) 0.5M AF+0.1N F, (7) 0.5M AF+0.5N F, (8) 0.5M AF+1N F, (9) 1M AF +1N F, (10) 1M AF+2N F

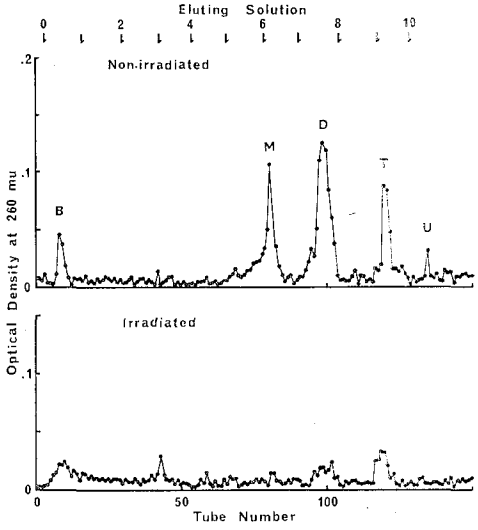

Fig. 2. Chromatographic patterns of the guanine compounds obtained from GTP material.

is shown in Fig. 2 and Tables 1 and 2. The guanine compounds obtained through the column were more sensitive to radiation than the adenine compounds as mentioned above. This may be caused by the difference of molecular structure between adenines and guanines. But a similar effect of irradiation for the adenosine nucleotides was observed in the guanine ones. That is, the rate of irradiation damage was high in the order of fractions M, D and T. The absorption spectra of all the fractions in this case remained unchanged before and after irradiation; absorption maximum at about 254 mμ.

2) **Pyrimidines**

When CTP and UTP aqueous solutions were also examined in the same manner as purines mentioned above, it was found that pyrimidines were broken more easily than purines by gamma irradiation with the dose of 2×10^5 R, and that cytosine compounds were more resistant than uracil compounds in small degree to the action of the radiation (Figs. 3 and 4 and Tables 1 and 2). Furthermore, the cytidine nucleotides were radiosensitive in proportion to their molecular weight. This was in contrast with the case of purine nucleotides mentioned above. But further investigation must be made on this point, because the degree of irradiation damage is too high to evaluate the effect of irradiation on these compounds.

On the other hand, no regularity was recognized in the irradiated uridine nucleotides (Table 2). From Table 2, two kinds of common features were deduced between adenines and cytidines, and between guanines and uracils. (1) fraction B of the former group increased in quantity after irradiation, but that of the latter one decreased, and (2) the molecules of the former compounds have no or less hydroxyl group (−OH) than those of the latter ones.

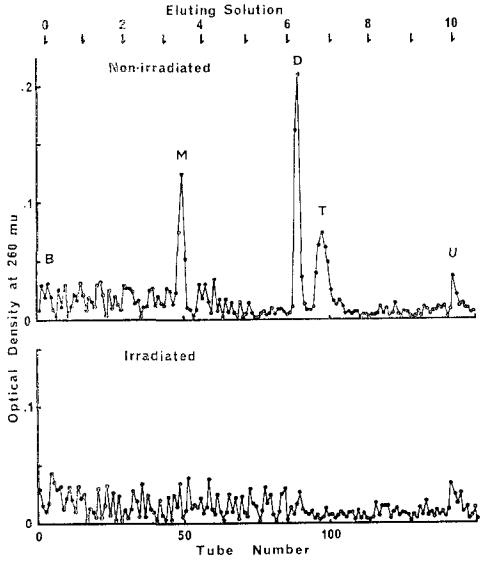

Fig. 3. Chromatographic patterns of the cytosine compounds obtained from CTP material.

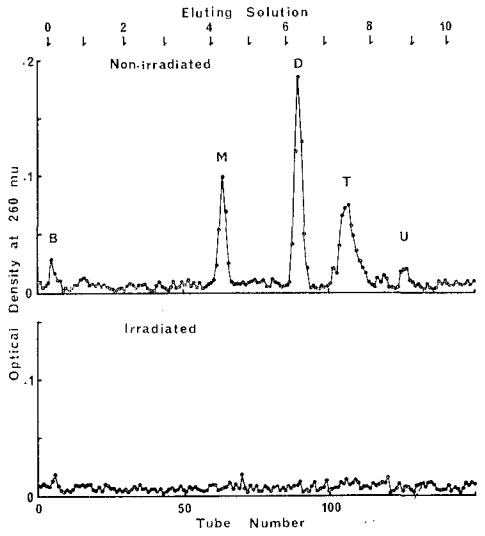

Fig. 4. Chromatographic patterns of the uracil compounds obtained from UPT material.

DISCUSSION

The release of free adenine from irradiated ATP was pointed out by Vaisey and Thatcher.[3] A similar result was also obtained from the present study. This suggests that the bond between adenine and its phosphate group was affected by gamma irradiation comparatively easily. The increase of fraction B of the irradiated ATP may be caused by the following reasons; adenine itself was considerably resistant to the action of gamma irradiation. Further, the released adenine was larger in quantity than that destructed by irradiation. Some results similar to this were also recognized in fractions B and U of irradiated CTP.

There was a significant difference of radiosensitivity between adenosine and guanosine nucleotides, and slightly between cytidine and uridine nucleotides. That is, adenines were resistant to the irradiation than guanines, and cytosines than uracils. This may be caused rather by the difference of molecular structure than by the difference of the molecular weight. The difference of molecular structure among the nucleotides corresponds to that among their base components. No hydroxyl group ($-OH$) is found in adenine, but one is present in guanine and cytosine, and two in uracil. On the other hand, amino group ($-NH_2$) exists in each of adenine, guanine and cytosine, but is absent in uracil. According to the data of chemical thermodynamics[13,14], the increase of entropy of these substances is slightly larger (about 0.2 kcal/mol) in the compounds bound with hydroxyl group than in those bound with amino group. In addtion to this, the enthalpy of total bonds of hydroxyl group is considerably lower than that of amino group. Therefore, the hydroxyl group within a compound is considered to be thermodynamically more unstable than amino group.

Accordingly, it may be concluded from the results of the present study that the order of radiosensitivity of purines and pyrimidines may be proportional to the number of hydroxyl group of the compounds used. A compound which has no hydroxyl group is more resistant to irradiation than that of one hydroxyl group, and a compound of one hydroxyl grop is more resistant than that of two, and so forth.

It can also be observed that the purine nucleotides were proportionally resistant to the irradiation according to the number of phosphoric acid residues. This fact suggests a conclution that, among the same basic nucleotides, the larger is the enthalpy of total bonds of nucleotide, the more resistant to ionizing irradiation. An explanation similar to this may be also applicable to the difference of radiosensitivity between purines and pyrimidines.

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REFERENCES

(1) S. Matsuoka, *Bull. Inst. Chem. Res., Kyoto Univ.*, **44**, 57 (1966).
(2) S. Matsuoka, in preparation
(3) E. B. Vaisey and F. S. Thatcher, *Radiation Research*, **17**, 74 (1962).
(4) G. Hems, *Radiation Research*, **13**, 777 (1960).
(5) C. Ponnamperuma, R. M. Lemmon and M. Calvin, *Radiation Research*, **18**, 540 (1963).
(6) E. S. Guzman Barron, P. Johnson and A. Cobure, *Radiation Research*, **1**, 410 (1954).
(7) R. L. Sinsheimer, *Radiation Research*, **1**, 505 (1954).
(8) R. L. Sinsheimer, *Radiation Research*, **6**, 121 (1957).
(9) W. E. Cohn and E. Volkin, *J. Biol. Chem.*, **203**, 319 (1953).
(10) S. Matuoka, *Acta Schol. Med. Gifu.*, **10**, 16 (1962).
(11) W. Cohn and C. E. Carter, *J. Am. Chem. Soc.*, **72**, 4273 (1950).
(12) D. H. Marrian, *Biochim. Biophys. Acta*, **12**, 492 (1952).
(13) L. Pauling, "The Nature of the Chemical Bond," Cornell Univ. Press (1960).
(14) R. Fujishiro, "Elementary Chemical Thermodynamics (Japanese)," Kyoritsu Shuppan Co. Ltd. Tokyo (1967).