Biophysical Aspects of Auger Processes, AAPM Symposium Series No. 8 (1992) 024-036
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DAMAGE TO ADENOSINE-TRIPHOSPHATE INDUCED BY MONOCHROMATIC X RAYS AROUND THE K SHELL ABSORPTION EDGE OF PHOSPHORUS

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ABSTRACT

Adenosine-triphosphate (ATP) is well known to have an important role in the energy metabolism in biological systems. The purpose of this study is to clarify the radiation effects on ATP specific to inner shell ionization. ATP, in concentrated aqueous solution, was irradiated with monochromatic X rays having energies of the resonance absorption peak of the phosphorus K shell, 2.153 keV, and slightly below and above the peak, 2.146 keV and 2.160 keV, selected from synchrotron radiation. Adenine, Adenosine 5'monophosphate (5'AMP) and Adenosine 5'diphosphate (5'ADP) were obtained as radioproducts by the method of high performance liquid chromatography

(HPLC). G values of these products were calculated on the basis of the absorbed energy. When the ATP solution of 0.282 mol/l was irradiated with 2.160 keV X rays which can ionize the K shell of phosphorus, G values of Adenine, 5'AMP and 5'ADP were estimated to be 1.4, 0.40 and 0.46, respectively. These values were respectively 1.3, 2.9 and 3.8 times higher than those obtained upon irradiation with 2.146 keV X rays which cannot ionize the K shell of phosphorus. These energy dependent enhancements may reflect the difference in energy absorption processes, especially the Auger cascade in phosphorus may be suspected to play an important role in these enhancements. With 2.153 keV X rays a stronger enhancement was obtained than for the irradiation with 2.160 keV. Radioproducts induced by the irradiation with ^{60}Co γ rays were also analyzed. In this case insignificant quantities of 5'AMP and 5'ADP were obtained and the G value of the Adenine produced was smaller than that for irradiation with X rays around the K absorption edge of phosphorus. This fact may suggest that the mechanism of radiation action on ATP induced by ^{60}Co γ rays is different from that by X rays around the K absorption edge of phosphorus.

INTRODUCTION

In order to determine the enhanced damage due to the Auger cascade initiated by the ionization of the K shell, the effects of irradiation on various systems with monochromatic X rays around an absorption edge of a selected component atom have been investigated. In particular, numerous studies which used bromine as a selected component atom have been tried. For example, the enhanced damage due to irradiation with monochromatic X rays having ionization energy of the K shell in bromine were examined in bromodeoxyuridine incorporated cells (1-6), plasmid DNA (4,7) or brominated synthetic DNA constituents (8,9). Referring to other selected component atoms, Shinohara *et al.* (10) have studied the sensitivity of HeLa cells with iododeoxyuridine incorporated into DNA to monochromatic X rays with energies slightly above the K shell absorption edge of iodine. In the situations of these experiments bromine and iodine are incorporated in the base moiety of DNA. On the other hand, phosphorus atoms are located at strategic positions as a constituent element of phosphodiester bonds of DNA. The irradiation with soft X rays around the K shell absorption edge of phosphorus in DNA may be considered to cause serious damage to DNA.

Recently some studies of the effects of irradiation with monochromatic X rays around the K shell absorption edge of phosphorous have been investigated using synchrotron radiation at the National Laboratory for High Energy Physics in Tsukuba, Japan. Plasmid DNA (11), chromosomes (12), T1 phage (13) and yeast cells (14) were irradiated with monochromatic X rays around the K shell absorption edge of phosphorus, and Auger enhancement effects induced via the ionization of the K shell of phosphorus were studied. In this report damage to ATP, especially the production of Adenine, 5'AMP and 5'ADP from ATP induced by monoenergetic X rays around the K shell absorption edge of phosphorus were studied by the method of HPLC.

MATERIALS AND METHODS

Preparation of Sample Solutions

Adenosine 5'-triphosphate (ATP) was purchased in powder form from Sigma Co. Ltd. It was dissolved in deionized triply distilled water without further purification and was used as a substance for irradiation. The concentration of ATP was 155 mg/ml (0.282 mol/l), and the pH of the ATP solution was 2.85. The ATP film for the measurements of the absorption spectrum was prepared by pressing the powder sample to 6.45 mg/cm^2 thickness between a pair of mylar films of 5 μm thickness. Adenine, Adenosine, 5'AMP and 5'ADP, purchased from Sigma Co. Ltd., were used as reference samples in the analysis of radioproducts.

Sources of Radiation

Synchrotron radiation from the 2.5 GeV electron storage (the Photon Factory, National Laboratory for High Energy Physics, Tsukuba, Japan) was used as the source of soft X rays. Details of the irradiation system, the characteristics of the X rays and their performance have been described elsewhere (15). White SR was deflected 2° upward in order to cut the high energy X ray components and was monochromatized with an InSb double-crystal monochromator. The full width at half maximum at 2 keV was about 1 eV. Irradiation with ^{60}Co γ rays was performed at the National Cancer Center Research Institute, Tokyo, Japan, using the irradiation apparatus with 24,000 Ci ^{60}Co γ source (Gamma-cell 220, AECL Industrial Irradiators).

Measurement of the Absorption Spectrum of ATP and Selection of X ray Photon Energy for Irradiation

As shown in Fig. 1, the absorption spectrum from the thin film of ATP was measured in the range of energy 2.10 - 2.25 keV, which is the soft X ray region around K absorption edge of phosphorus. For spectrum measurement, an ATP film was inserted between the monochromator and the ionization chamber installed downstream of the beamline. The absorption spectrum showed a stepwise change, going from below to above the edge with fine structure of a sharp peak at around 2.153 keV. A sharp absorption peak was found to be characteristic of the structure of P surrounded by O atoms in the form of PO_4^{3-} (14, 16). In the present study irradiations were carried out with monochromatic X rays of 2.153, 2.146 and 2.160 keV. The 2.153 keV X rays (K X ray (P_O)) energy corresponds to the resonance peak and 2.146 keV X rays (K X ray (P_-)) and 2.160 keV X rays (K X ray (P_+)) correspond to slightly below and above the peak energy, respectively.

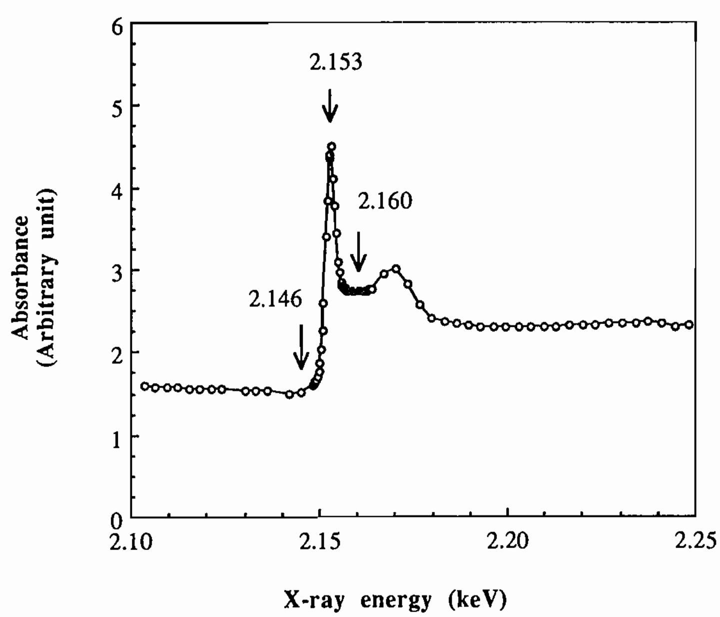

FIG. 1. Absorption spectrum of ATP film around the K absorption edge of phosphorus.

Irradiation Techniques

Monochromatic X rays were transported through a 5 μm mylar film into an atmospheric sample chamber installed at the end of the vacuum line (Beamline 11 B of Photon Factory). At the sample chamber a specially designed cell made of acrylic resin (Fig. 2) was used to hold the ATP solution for irradiation. The cell had a mylar film window of 5 μm thickness, through which the sample was exposed to X rays. The area of the window was 5 mm x 10 mm and the cell depth was 2 mm. The X ray beam size at the sample position was 4 mm x 8 mm. Under irradiation, ATP solutions were stirred with a glass-coated piece of iron. Irradiation was carried out under aerobic conditions at 23 ± 2°C. A free air parallel-plane ionization chamber was used for measurements of the exposure rate. The chamber was placed in front of the sample position. The exposure rate was 15.5 C/kg per min at a ring current of 250 mA. During the irradiation with ^{60}Co γ rays, 200 μl of the ATP solution was sealed in a 20 cm^3 glass ampoule under aerobic conditions. The exposure rate, measured with a Fricke actinometer, was 4.5 kGy/h.

Estimation of Absorbed Dose and Calculation of G value

When photons having energies around the K absorption edge of phosphorus are transmitted through water, the path length of water needed to reduce the intensity of photons to 1/e is very thin such as 20 μm (17). Therefore all the incident photons were absorbed completely by the ATP solution located in the cell with 2 mm depth. The estimation of the absorbed dose is rather simple for this situation. In this case all energies up to the maximum photon energies passing through the mylar film of the cell may be used to estimate the absorbed dose. The photon fluence Φ (photons/m^2) per 1 C/kg was calculated using:

$$\Phi = W \times 1/E \times 1 / (\mu_{en}/\rho)_{air} \qquad (1)$$

where $(\mu_{en}/\rho)_{air}$ is the mass energy absorption coefficient of air, W the energy required to produce an ion pair in air at room temperature (33.7 J/C), and E the energy of an X ray photon. In the present study, G values, which are defined as the number of radioproducts in an irradiated solution per absorbed energy of 100 eV, were calculated on the basis of the absorbed dose estimated as described above.

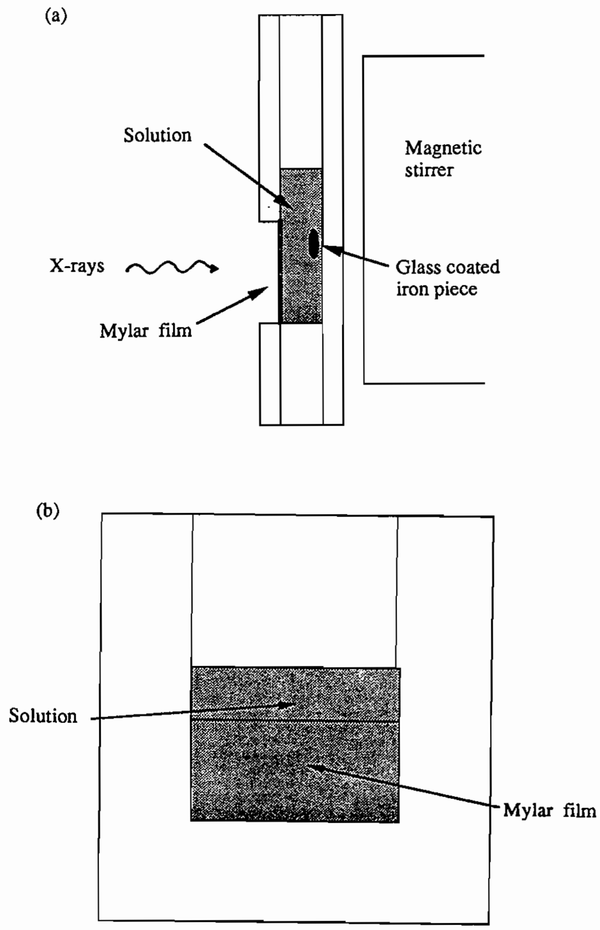

FIG. 2 A view of the cell prepared for irradiation with X rays around the K absorption edge of phosphorus. a) Side view of the cell. b) Front view of the cell. Scale (1 in = 8 mm).

Analysis of Radioproducts

The analyses of radioproducts were performed by HPLC using a Waters Model ALC/GPC 609G. An anion exchange column 10 mm in diameter by 100 mm in length (Partisil SAX) was employed. Two kinds of buffer solutions were used in the moving phase; A) a solution of 0.05 mol/l KH_2PO_4 (pH 4.5), B) a solution of 0.25 mol/l KH_2PO_4 with 0.5 mol/l KCl (pH 5.0). Buffer solutions of A and B were delivered at linear gradient from 100% A to 100% B for 10 min and then 100% B was delivered for 40 min. The flow rate was 3 ml/min throughout the measurements. The reverse-phase column (μBondapak C_{18}) was used to get supplementary information and in this case a buffer solution of A was delivered at 3 ml/min. The spectra were monitored optically at 260 nm. The chromatograph was operated at a temperature of 21 ± 1°C. Adenine, Adenosine, 5'AMP and 5'ADP were used as reference samples. The retention times were 1.80 min for Adenine, 1.70 min for Adenosine, 2.95 min for 5'AMP and 9.30 min for 5'ADP during the ion exchange analysis.

RESULTS

HPLC elution profiles of ATP irradiated with K X rays ($P_{-,o,+}$) were observed (Figures are not shown). It was thus found that there were no qualitative differences among these HPLC patterns. The main radioproducts, Adenine, 5'AMP and 5'ADP, were identified among several fractions in the HPLC profiles, judging from the retention times of the reference samples described in the MATERIALS AND METHODS section above. By the method of reverse-phase HPLC it could be verified that Adenosine was not obtained as one of the radioproducts. The amount of each radioproduct was estimated from the peak area from each respective signal on the elution pattern and was plotted against absorbed dose. The amount of produced Adenine, 5'AMP and 5'ADP from ATP irradiated with K X ray (P_o), expressed in terms of the number of those radioproducts produced per unit volume of irradiated ATP solution of 1 cm^3, were plotted as a function of absorbed dose in Fig. 3. These were seen to increase linearly with increasing absorbed dose. Also during irradiation with K X rays (P_-) and (P_+), the amount of produced Adenine, 5'AMP and 5'ADP increased linearly with increasing absorbed dose (Figures not shown). G values of these radioproducts calculated on the basis of the absorbed dose, which was described in the MATERIALS AND METHODS section above, are shown in Table I for various cases. In Fig. 4, G values of Adenine, 5'AMP, and 5'ADP are shown as a function of the incident photon energy. As shown in Fig.4, the G values of these radioproducts upon irradiation with K X rays (P_o) and (P_+) were much larger than those from K X ray (P_-). Details about these enhancement effects will be described in the DISCUSSION.

The dependence of the amounts of produced Adenine, 5'AMP and 5'ADP on the absorbed dose in the case of irradiation with ^{60}Co γ rays are

TABLE I

G Values of Radioproducts from ATP Irradiated with X rays and ^{60}Co γ rays

Substrate	Radio-products	2.146 keV X rays	2.153 keV X rays	2.160 keV X rays	$\frac{G_{2.153\,keV}}{G_{2.146\,keV}}$	$\frac{G_{2.160\,keV}}{G_{2.146\,keV}}$	^{60}Co γ rays
	Adenine	1.1	1.6	1.4	1.5	1.3	0.88
ATP	5'AMP	0.14	0.48	0.40	3.4	2.9	0.00
	5'ADP	0.12	0.47	0.46	3.9	3.8	0.00

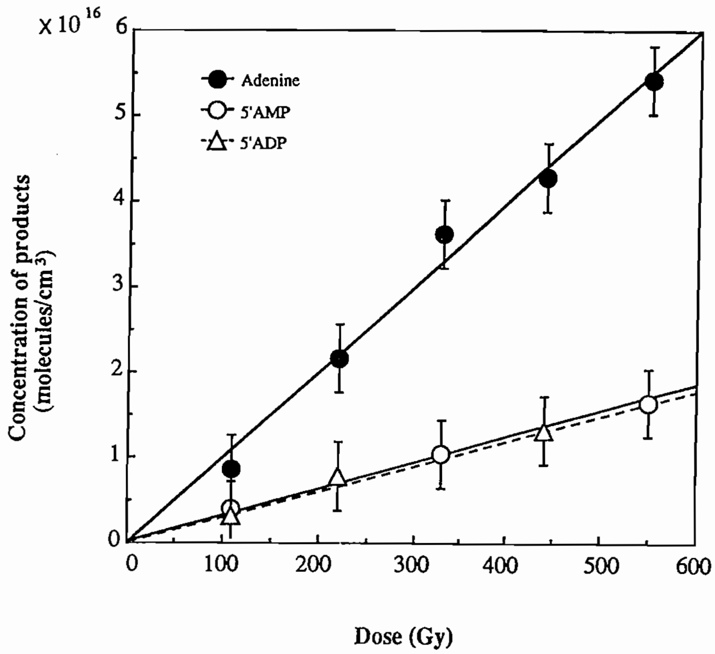

FIG. 3. Dose dependence of the concentrations of Adenine, 5'AMP and 5'ADP in the ATP solution following irradiation with 2.153 keV X rays (K X ray (P_o)).

shown in Fig. 5. Linear production of produced Adenine with increasing absorbed dose was obtained, however, 5'AMP and 5'ADP were not significantly produced. The G values of these products are shown also in Table I.

DISCUSSION

In this report only Adenine, 5'AMP and 5'ADP were analyzed as main radioproducts, although lesser amounts of other products were seen in the ion exchange liquid chromatogram. As shown in Table I, K X rays (P_0) and (P_+) effectively induced the production of Adenine, 5'AMP and 5'ADP, relative to K X ray (P_-). Irradiations with K X ray (P_+), which can ionize at the K shell in phosphorus atom, gave 1.3, 2.9 and 3.8 times higher G values for Adenine, 5'AMP and 5'ADP, respectively, compared with K X ray (P_-) which can not ionize the K shell of phosphorus. Upon irradiation with K X ray (P_0),

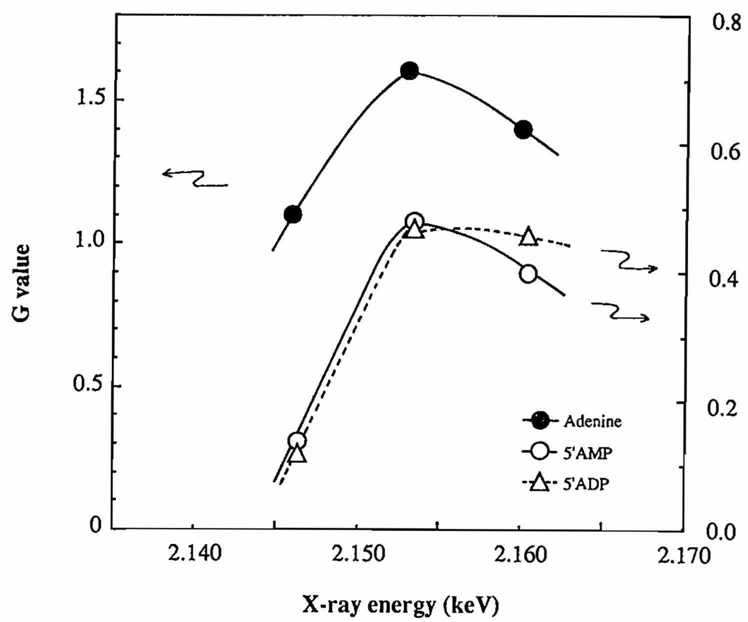

FIG. 4. The dependence of the G values of Adenine, 5'AMP and 5'ADP on the incident photon energy.

which is just the resonance energy of the K absorption edge of phosphorus, the maximum G values for these radioproducts were obtained for all three X ray energies. From this experiment it can be said that a slight increase in photon energy, for example only 0.3% increase {(2.153 - 2.146) / 2.146}, causes a greatly enhanced ratio of damage to ATP such as 45% (1.6 / 1.1 - 1) or 290% (0.47 / 0.12 - 1). The reason for this great enhancement in damage is suggested to be as follows. The photoelectric absorption of photons having energies slightly above the K absorption edge is followed by the Auger cascade and leaves the atom highly ionized. Further, both the low energy Auger and Coster-Kronig electrons are set in motion, and the excitation energy released in charge neutralization is capable of producing extensive bond rupture and damage. The photons having energies slightly below the K edge do not induce these physical processes caused through the initiation of ionization at the K shell in phosphorus. The enhancement ratios for production of 5'AMP (3.4 for K X ray (P_O), 2.9 for K X ray (P_+)) and 5'ADP (3.9 for K X ray (P_O), 3.8 for K X ray (P_+)) were larger than those for Adenine (1.5 for K X ray (P_O), 1.3 for K

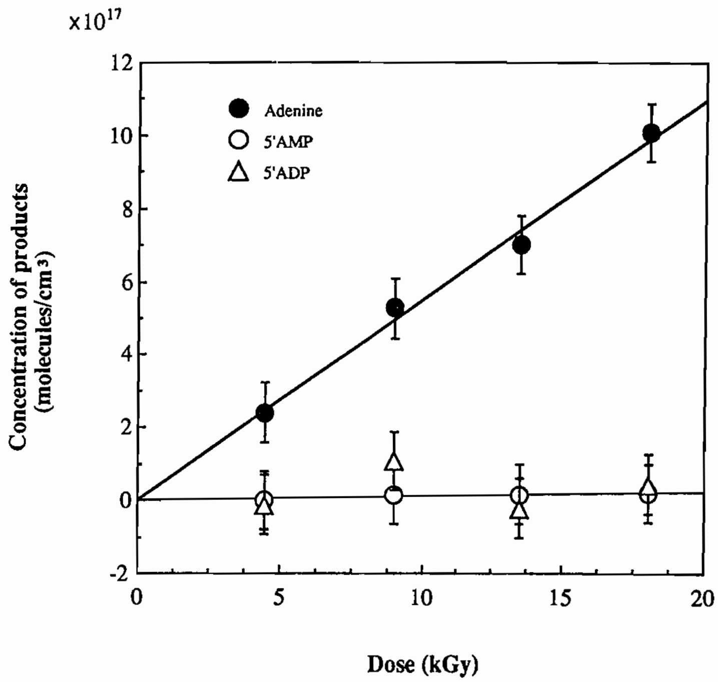

FIG. 5. Dose dependence of the concentrations of Adenine, 5'AMP and 5'ADP in the ATP solution irradiated with ^{60}Co γ rays.

X ray (P_+)) when comparing the G values upon irradiation with the K X rays (P_O) and (P_+) to that of K X ray (P_-). The reason for this phenomenon may be due to the release of electrons from the K shell followed by the positive ionization of the phosphorus atom. This then causes the production of inorganic phosphate which is more sensitive than the production of Adenine located at the base position because it is farther from the phosphorus atom.

The physical processes following the photoelectric absorption of photons having energies in the resonance region of the K edge should be carefully considered. In this experiment indirect effects from water should not be disregarded. From a simple calculation using the mass energy absorption coefficients (17), weight, and volume of water and ATP, it is estimated that about 85% of the total energy is absorbed by the water in the ATP solution during irradiation with K X ray (P_-), and 65% and 76% for K X rays (P_o) and (P_+), respectively. That is, the indirect contributions via water to the G values are reduced to about 80 or 90% during the irradiation with K X rays (P_o) and (P_+) compared with that of K X ray (P_-). This fact means that indirect effect via water has no essential role in the enhancement of G values of radioproducts during the irradiation with K X rays (P_o) and (P_+).

In this experiment irradiation of ATP solution with ^{60}Co γ rays was performed as a reference. Although the chromatogram pattern observed when ATP was irradiated with ^{60}Co γ rays was similar to that with K X rays ($P_{-,o,+}$), the corresponding G values of produced Adenine, 5'AMP and 5'ADP were very different. The G value of produced Adenine upon irradiation with ^{60}Co γ rays was 0.88 as shown in Table I. This value is much smaller than that obtained upon irradiation with K X rays ($P_{-,o,+}$). Another difference is that 5'AMP and 5'ADP were not significantly produced during irradiation with ^{60}Co γ rays, whereas they were clearly observed upon irradiation with K X rays ($P_{-,o,+}$). Thus the probability of production of intact Adenine, 5'AMP and 5'ADP is higher for irradiation with K X rays ($P_{-,o,+}$) than for ^{60}Co γ rays. It is suspected that during irradiation with ^{60}Co γ rays a higher possibility of degradation of sugar accompanying the production of Adenine, 5'AMP and 5'ADP is apparently found than during irradiation with X rays around the K absorption edge of phosphorus. Further studies to clarify the sugar damage to ATP induced by irradiation with X rays around the K absorption edge of phosphorus should be carried out.

ACKNOWLEDGMENTS

We would like to express our thanks to Dr. N. Munakata of the National Cancer Center Research Institute for his help in using the irradiation system with ^{60}Co γ rays. We greatly appreciate Mr. A. Yokoya and Ms. N. Usami for their technical instruction and assistance throughout the irradiation experiments. This work was performed under the approval of the Photon Factory Advisory Committee (Proposal No. 88-153)

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DISCUSSION

Adelstein, S. J. Were you able to measure G values for the destruction of ATP *i.e.* G(-ATP) as well as for the formation of adenine, AMP and ADP?

Takakura, K. It is difficult to detect the reduction in the amount of ATP because the ratio of the reduction must be between about 0.1% to 1%. On the other hand, small amounts of radioproducts such as adenine, AMP, and ADP can be detected if the amounts of these in the control are not so much. The corresponding signals in HPLC can be observed as newly produced signals.

Goodhead, D. It seems interesting that you find the two 5' products produced even below the phosphorus K edge but that they are not produced by ^{60}Co γ rays. Does this suggest even an L shell absorption in phosphorus (without a consequent Auger cascade) can produce a 5' product? Yet ^{60}Co γ rays do not even though secondary-electron ionization must take place (occasionally and randomly) in the phosphorus L-shell. Is this because there are so few such ionizations from ^{60}Co γ rays?

Takakura, K. I found that the two 5' products are produced even below the phosphorus K edge. However, in the case of irradiation with ^{60}Co, the γ rays produce the two 5' products but further degradation of the sugar attached to the 5' products apparently leads to nearly zero production of these products.

Martin, R. I presume that the ^{60}Co γ ray/ATP experiment is not new, and that there is established data on the radiation chemistry of DNA. What is known about the mechanism of adenine formation from ATP?

Takakura, K. I was looking for the publications concerning radiation effects on ATP using ^{60}Co γ rays but I didn't find them. Would you please tell me the references concerning this if you know of them? I am sorry, but I cannot tell you the exact mechanism of adenine formation from ATP. I think ESR would give the answer.