32P-retention in DNA Irradiated in a Reactor.

Effects of the Various Solvents on the 32P-retention

KAWAI*, Kenichi and Mitsuhiko AKABOSHI*

(Received, May 1, 1971)

ABSTRACT

32P-retention in DNA was enhanced with increasing irradiation time when wet DNA was irradiated at -78°C. This suggested an extreme complexity of the mechanism underlying the retention. Thus, the effects of various solvents on the retention were examined as an approach to the elucidation of the mechanism of 32P-retention. When DNA was irradiated with alcohols, 32P-retention in DNA was decreased markedly. This suggests that hot 32P atoms were caught by alcohols or alcohol-originated radicals to yield the acid soluble fraction. From the experiments in which the other organic or inorganic compounds were added, the mechanism of 32P-retention in DNA was discussed.

INTRODUCTION

Although the elucidation of the chemical effects of the neutron capture reaction on DNA is very important to analyse its effects on cells or tissues in a living organism, little is reported about the effects on DNA or DNA related substances except a few cases.1,2) As shown in the previous paper3), when DNA was irradiated in a reactor, about half of the 32P derived from the nuclear reaction (n, γ) was attached to original DNA molecule in the form vulnerable to the action of the phosphatase, viz., in monoester form. This high retention of 32P in DNA molecule as compared with that in the other compounds4–6) is very interesting. Thus, the next work7) by us, designed to increase the 32P-retention further, showed that 32P-retention in DNA could be enhanced with increasing irradiation time when wet DNA

* Research Reactor Institute, Kyoto University, Kumatori-cho, Sennan-gun, Osaka, Japan.
was irradiated at dry-ice temperature. In the present paper, the results of further investigations to define the conditions necessary for the enhancement and the effects of the various solvents on $^{32}$P-retention in DNA are described.

**MATERIALS AND METHODS**

One mg of Salmon sperm DNA (supplied by California Biochemical Co.) was sealed into a polyethylene vessel with various solvent (0.01 ml, unless otherwise stated). The vessel was put into a pneumatic capsule with or without powdered dry-ice, and was irradiated in a pneumatic tube of KUR (Kyoto University Reactor) for 15-600 sec with a neutron flux of approximately $5 \times 10^{12}$ n.cm$^{-2}$ sec$^{-1}$. After the irradiation, samples were treated according to Sibatani's method with minimal modification, and three fractions (DNA, acid soluble and orthophosphate) were obtained. The radioactivity of $^{32}$P in each fraction was counted with a lead shielded low-back GM-counter up to 1000 counts. Individual experiment was repeated three times and the values were averaged. Details of the experimental procedures were described in the previous paper.

**RESULTS**

1. **$^{32}$P-distribution in each fraction of DNA irradiated under the various conditions**

   Results obtained from the irradiation of dry or wet DNA (1 mg DNA in 0.01 ml H$_2$O) at reactor temperature (supposed to be 60-70°C) or dry-ice temperature were shown in Fig. 1. When dry or wet DNA was irradiated at reactor temperature, $^{32}$P-distribution in each fraction was almost constant against irradiation time, although in the case of wet DNA, $^{32}$P-retention in DNA fraction was decreased to about 20% due possibly to decomposition of DNA molecules. However, when the irradiation was carried out at dry-ice temperature $^{32}$P-retention was enhanced with increasing irradiation time, and the enhancement was more marked in wet DNA than in dry DNA. $^{32}$P-activity in orthophosphate fraction was approximately doubled by the presence of H$_2$O in the both conditions of irradiation, i.e., at reactor and dry-ice temperature.

2. **$^{32}$P-retention in DNA irradiated with various alcohols**

   To seek a chemical nature of hot $^{32}$P atom, 1 mg of DNA was irradiated with various concentration of alcohols (methanol, ethanol and propanol) for 10 min at dry-ice temperature, and the $^{32}$P-distribution in each fraction was determined (Fig. 2). Fig. 2 shows the changes in the $^{32}$P-distribution in each fraction as a function of the concentration of the alcohols. $^{32}$P-retention in DNA fraction was decreased with increasing concentration of the alcohols; initially, rapidly, but rather slowly thereafter. However, at high concentration of alcohols, the $^{32}$P-retention began to increase again to the original level. The point at which the retention begins to increase differed with different alcohols. (About 70% in the case of methanol, 60% in ethanol and 50% in propanol, namely, the higher the alcohol, the point shifted
to the left). $^{32}$P activity in the orthophosphate fraction was almost constant at the every concentration of alcohols. Thus, the variation of $^{32}$P-distribution seems to depend on the exchange of $^{32}$P or $^{32}$P-containing fragments between DNA and acid soluble fraction.

3. Dilution effects

To seek the nature of the chemical reactions between $^{32}$P and the solvent molecules, 1 mg of DNA was irradiated with different amount of ethanol the suitable concentration of which was selected from the above experiments (Fig. 3). When DNA was diluted in H$_2$O and irradiated for 10 min at dry-ice temperature, $^{32}$P-retention in DNA fraction was decreased with dilution while $^{32}$P-activity in acid soluble and orthophosphate fraction was increased slightly. This showed that the probability of reaction of the hot $^{32}$P atom with OH radical originated from H$_2$O was increased with dilution. However, when DNA was diluted with ethanol and irradiated, $^{32}$P-retention in DNA fraction was decreased markedly with dilution, even in the
low concentration of ethanol (5% ethanol, Fig. 3-b). This suggested that in the presence of ethanol, coming hot $^{32}$P atoms were caught easily by ethanol or ethanol-originated activated molecules to yield acid soluble fraction. Thus, it is reasonable to find that $^{32}$P activity in orthophosphate fraction was decreased to about 10% as compared with about 25% in the case of H$_2$O dilution. When DNA was irradiated with 100% ethanol, dilution effect could not be observed. Considering that DNA molecule did not dissolve in the absolute ethanol, these results were reasonable. However, a high $^{32}$P-activity in orthophosphate fraction in this case could not be explained.

4. Effects of the other organic and inorganic compounds

Dilution effects by DNA-related compounds such as cytidine and d-ribose, and inorganic C-compound (sodium bicarbonate), were examined in comparison with the effects of alcohol. Among the results obtained with various concentrations of these compounds, suitable concentrations corresponding to 5% ethanol were picked up in the Fig. 4. Almost the same results as in the case of alcohols were obtained with the organic compounds. Namely, the effects of ethanol could be reproduced by corresponding concentrations of cytidine or d-ribose. This showed that the action of
alcohols to decrease $^{32}$P-retention in DNA was due simply to competitive reaction. However, in the case of sodium bicarbonate, decrease of $^{32}$P-retention by dilution was not marked. This showed that the competitive reaction occurs less in the case of an inorganic compound.

**DISCUSSIONS**

When DNA was irradiated at $-78^\circ$C, $^{32}$P-retention in DNA was enhanced with increasing irradiation time. This shows that the process of $^{32}$P-retention has certain
life at low temperature, and yet, in order to attain the chemical reactions the species concerned need the activation process by some agent in reactor (γ-rays, fast neutrons etc.). H₂O may, becoming radicals, play some important role in this recombination reaction. Since the enhancement could not be observed in the other treatments such as prolonged storage at -78°C or heat (100°C)⁹, it should be defined as a radiation annealing reaction.

When DNA was irradiated with various concentration of alcohols, ³²P-retention in DNA was decreased markedly, but at the high concentration of alcohols, it was increased again to the original level. The point at which ³²P-retention begins to increase differed according to the kinds of alcohols added. This may be due to different solubility of DNA in these alcohols. When DNA was diluted with ethanol,
and irradiated, $^{32}\text{P}$-retention in DNA was decreased with dilution even in the low concentration of a few per cent, while $^{32}\text{P}$-activity in acid soluble fraction was increased. This finding may indicate that the hot $^{32}\text{P}$ atoms originated from DNA were caught easily by alcohols or alcohol originated active molecules in the competition with target molecule (DNA). Since the concentration of DNA of 1 mg/0.01 ml solution (about 0.3 Mol, corrected to nucleotide unit) correspond to the those of the additives, the rate of competition of target molecules and the additives for $^{32}\text{P}$ or $^{32}\text{P}$-containing fragments seems to be roughly equivalent to each other. The reasons that only the inorganic compound, sodium bicarbonate, showed dissimilar behaviour can not be explained now.

ACKNOWLEDGEMENTS

The authors thank Prof. S. Iwata and Prof. Y. Kiso for their kind advices and discussions, and Miss Y. Tanaka and Miss H. Naka for their technical assistance.

REFERENCES

1. Halmann M. and I. R. Miller (1963) Thermal neutron capture in crystalline deoxyribo-nucleic acid. Biochim. Biophys. Acta, 72, 483-487.
2. Mautner, H. G., Donelly, B., Lee, C. M. and G. W. Leddicotte (1962) Neutron activation as a method for labelling the phosphorus of nucleotides. J. Am. Chem. Soc., 84, 2021-2022.
3. Akaboshi, M., Maeda, T. and A. Waki (1967) The chemical form of $^{32}\text{P}$-compounds after nuclear recoil reactions in neutron-irradiated nucleotides and DNA. Biochim. Biophys. Acta, 142, 258-291.
4. Campbell, I. G., Poczynajlo, A. and A. Siuda (1959) The hot atom chemistry of organic phosphorus compounds. I. The products of neutron irradiation of tributyl phosphate. J. inorg. nucl. Chem., 10, 225-237.
5. Wheeler, O. H., Facetti, T. F. and M. Santos (1966) Neutron activation products from aromatic compounds. Puerto Rico Nuclear Center-101.
6. Kiso, Y., Kobayashi, M., Kitaoka, Y., Kawamoto, K. and J. Takada (1967) Hot atom chemistry of phenylphosphorus compounds. Bull. Chem. Soc. Japan, 40, 2779-2787.
7. Akaboshi, M., Kawai, K., Waki, A. and T. Maeda (1968) $^{32}\text{P}$-retention and physical change of DNA irradiated in a reactor. J. Rad. Res., 9, 100-104.
8. A. Sibatani (1959) *In vitro* incorporation of $^{32}\text{P}$ into nucleic acids of lymphatic cells.— Effects of X-irradiation and some other agents. Exptl. Cell Res., 17, 131-143.
9. Kawai, K., Waki, A., Akaboshi, M. and S. Iwata (1969) $^{32}\text{P}$-retention of DNA irradiated in a reactor. The enhancement of the retention with increasing irradiation time. J. Rad. Res., 10, 83-87.
10. Zahn, U. (1967) Recoil reactions in crystalline $\text{C}_2\text{H}_3\text{Mn(CO)}_3$. Radiocohimica Acta, 8, 177-178.