# Radiation Effect on the Nucleic Acid

Yasuo Imai\*

(Department of Physiology, Faculty of Medicine, Kyoto University)

Received December 28, 1962

In this paper, changes of magnetic properties of nucleic acid by  $\tilde{r}$ -ray or neutron irradiation are chiefly described. Diminution of the ferromagnetic property of native nucleic acid samples by irradiation in water media was observed by the ESR and static magnetic susceptibility measurements at the room temperature.

#### INTRODUCTION

On the ferromagnetic property of native nucleic acid samples and their magnetic centers some papers have already been published by Blumenfeld<sup>1)</sup>, Müller *et al.*<sup>2)</sup>, Shulman *et al.*<sup>3)</sup> and Rlois<sup>4)</sup>.

This ferromagnetic property is described by some one<sup>3)</sup> as the phenomena from paramagnetic impurities such as Fe<sup>+++</sup> or Fe<sup>++</sup>, Co<sup>++</sup>, Ni<sup>++</sup> or Cu<sup>++</sup>, while by others<sup>1)(2)(4)</sup> as the specific property from macromolecular nucleic acid itself.

We have measured the magnetic susceptibility and ESR spectra of nucleic acid. Newly prepared samples show much more strong magnetic value than those expected from paramagnetic iron concentration.

If the ferromagnetic property of nucleic acid is produced by the configuration of macromolecules, it would be diminished by destruction of the macromolecular compounds with irradiation or other destructive procedure.

# EXPERIMENTAL METHOD

Magnetic susceptibility was measured by a pendulum type magnetic balance<sup>5</sup> and conditions of ESR spectra obtained were X-band, low frequency magnetic field modulation, magnetic field sweep width (300 Gauss~6000 Gauss) and at the ambient air temperature. The samples irradiated by  $\gamma$ -rays with a total dose of 10<sup>5</sup> r and by neutrons with a flux of 10<sup>12</sup>/sec for one week duration. DNA was prepared from calf tymus by the Hammersten's method, and RNA from calf liver by the Schneider's method.

Irradiation of nucleic acids was made *in vacuo* in the presence of  $O_2$  and in water solution. Other destructive procedure was made by hydrolytic and enzymatic cleavage.

### RESULTS

When DNA samples freshly prepared were inserted in the inhomogeneous

<sup>\*</sup>今井安男

## Yasuo IMAI

magnetic field, the attracting for ceof hundreds dynes was produced and the concentration of the magnetic center was calculated approximately to be  $10^4$  spins per one molecule as the free radical electrons. This value suggests that one free electron would exist for each group of two or three nucleoside residues. The same values was also observed in RNA samples. And saturation

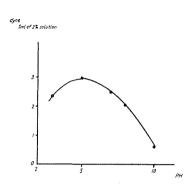


Fig. 1. Correlation between the magnetic susceptibility of nucleic acid and pH of solution.

Ordinate: attracting force in dynes of sample in the inhomogeneous magnetic field. Abscissae: pH values.

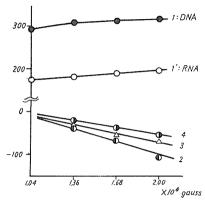
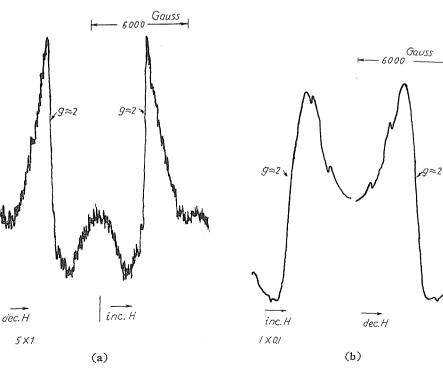


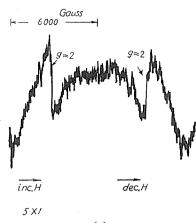
Fig. 2. Changes of magnetic susceptibility of completely damaged nucleic acids by irradiation, hydrolytic and enzymatic treatments. Ordinate: attracting force of samples in dynes. Abscissae: magnetic field strength.

1, 1') Native, 2) irradiated, 3) hydrolyzed nucleic acid and 4) damaged one with ribonuclease.



(98)

Radiation Effect on the Nucleic Acid



(c)

Fig. 3A. ESR spectra of native nucleic acids.

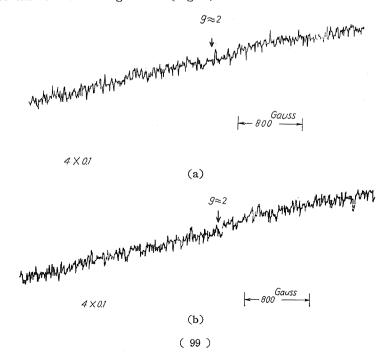
a) signals of RNA prepared from calf liber.

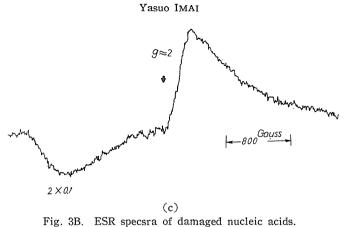
b) signals of DNA prepared from calf tymus.

c) signals of protein prepared simultaneously with the former (signals observed are the same as that of the sample tube).

phenomena of magnetic values was observed with the field strength of 16000 Gauss or more.

When the nucleic acids were dissolved in buffered solution, these magnetic phenomena were also observed, but the magnetic susceptibility was the function of the pH having a maximum at  $pH=5\sim6.5$  (Fig. 1), and this was reversible in the range of  $pH=3\sim10$ . For the samples of which polymer configulation destroyed completely by enzymatic and hydrolytic cleavage, the magnetic susceptibility was shifted to diamagnetism (Fig. 2).





a) signals of nucleic acid treated by HCl, 1/10N for 15 hrs.

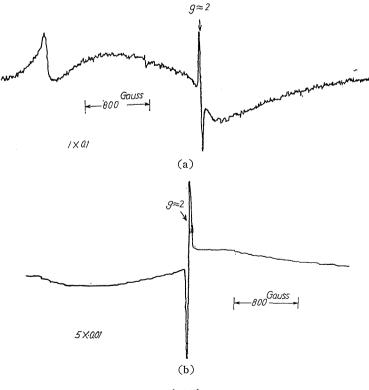
b) signals of nucleic acid treated by ribonuclease for 15 hrs.

c) control.

The magnetic value of ribosomal particles containing more iron impurity, obtained from calf liver by ultracentrifugation at  $4 \times 10^4$  g, was also diminished remarkably by ribonuclease treatment.

The ESR spectra of native nucleic acids are the same as those obtained by Blumenfeld *et al.*<sup>1~4)</sup> (Figs. 3a, b and c).

In the solid samples irradiated by  $\gamma$ -rays and neutrons the ESR spectra were not changed except for the signal of free radical produced by irradiation (Figs.



(100)

Radiation Effect on the Nucleic Acid

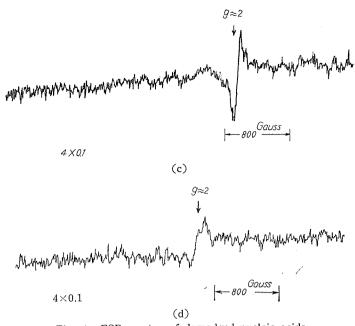


Fig. 4. ESR spectra of damadged nucleic acids.
a) signals of DNA irradiated by γ-rays (10<sup>5</sup> r) in solid phase.
b) signals of DNA irradiated by neutron (10<sup>12</sup>/sec) in solid phase.
c) signals of DNA irradiated by 10<sup>4</sup> r γ-rays in solution.
d) signals of DNA containing cystein irradiated by 10<sup>4</sup> r γ-rays in solution.

4a and b). While for the sample irradiated in the water media, the broad intensive signal could not be observed and the signals of free radicals produced by irradiation (Figs. 4c and d) were observed.

Proteins obtained as by-products simultaneously did not show such phenomena as nucleic acids in native state except for free radicals produced by the irradiation<sup>6)</sup>.

#### DISCUSSION

Although the ferromagnetic property of nucleic acids obserbed by ESR or static magnetic susceptibility measurements is not yet determined whether it be an original property itself or impurity ion, from the present experiment it may be said that this ferromagnetic property would be related to the macromolecular configulation of the nucleic acid.

For the fact why the nucleic acid is the only one that shows ferromagnetic property in the biological substances, charge transfer complexes between neighbouring nucleoside rings would be responsible<sup>718)</sup>. If this is true, the specificity, so called, of nucleic acid will lodge heavily on this ferromagnetic property as well as on the arrangement of nucleoside residues.

Irradiation effect on living things are described widely as a largely motal and partly genetical one. From the electric point of view, basic effect of irradiation on nucleic acid is the destruction forming free radicals. When the

### Yasuo IMAI

destruction is slight, few molecule would remain almost intact. The later one will be responsible for evolution.

From the biological and biophysical points of view, further experimental works should be necessary to get more informations on the function of the ferromagnetic property of the nucleic acid and its compounds in the living materials.

### REFERENCES

- (1) L. A. Blumenfeld and A. E. Kalmanson, Biophisika, 124, 1144 (1959).
- (2) A. Müller, G. Hotz and K. G. Zimmer, Biochem. Biophys. Res. Comm. 4, 214 (1961).
- (3) R. G. Shulman, W. M. Walsh, H. J. Williams and J. P. Wright, Biochem. Biophys. Res. Comm. 5, 52 (1961).
- (4) M. S. Rlois and J. E. Maling, Biochem. Biophys. Res. Comm. 4, 252 (1961).
- (5) Y. Imai, J. Biochem, in press.
- (6) Y. Imai, This Bulletin, 39, 138 (1961).
- (7) M. G. Evans and J. Gergely, Biochim. Biophis. Acta, 3, 188 (1949).
- (8) T. A. Hoffmann and J. Ladik, Cancer Res., 21, 474 (1961).