Note

¹H-NMR Study of the Effect of Gamma-ray Irradiation on the State of Cell Water in *Tetrahymena pyriformis*[†]

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The gamma irradiation effect on the state of water in Tetrahymena pyriformis was studied by ¹H-NMR. The spin-lattice relaxation time (T_1) value was measured, in which the transient spectra after 180° pulse were observed varying pulse interval (PI). This method revealed the appearance of another small peak of water together with the main peak of water in irradiated cells, whereas any small peak of water was not observed in unirradiated cells. The T_1 value of the small peak was short compared with that of the main peak, suggesting that the water corresponding to the small peak is different from that corresponding to the main peak.

Key Words: gamma-ray irradiation, *Tetrahymena pyriformis*, nuclear magnetic resonance, NMR, free water in cell, bound water in cell

1. Introduction

Nuclear magnetic resonance (NMR) has been used to study the behavior of water in biological cells and many correlations have been found between the NMR parameters of water and the physiological or pathological states of cells¹⁾. It is well-known that there are two states of water, free and bound (structured) water, in cells and the spin-lattice relaxation time (T_1) of the former is longer than that of the latter²⁾. An important development in the NMR studies of water in biological systems has been to show that the measurements of T_1 value of water in tissues or

organs have a potential use in the cancer research. Damadian et al.³⁾ have reported that T_1 values of all the tumors studied were significantly longer than those of the corresponding normal tissues, indicating the increase of free water in the cancer cells. On the other hand, Hazlewood et al.⁴⁾ have described the increase of structured water in developing muscle with regard to aging.

The ciliate protozoan *Tetrahymena pyriformis*, a free-living unicellular eukaryote, has most of organelles that are contained in mammalian cells. In this paper, we reported the effect of γ -ray irradiation on the state of water in *Tetrahymena pyriformis* using ¹H-NMR spectroscopy. The ¹H-NMR spectrum of water in cells is usually observed as a single peak. Using the (180° - τ - 90°) pulse sequence, the T_1 value of the peak is evaluated by a least-squares analysis of the experimental curve (inversion recovery method). How-

[†] ¹H-NMR によるテトラヒメナ細胞中の水の状態に対する γ 線照射の効果についての研究。五島廉輔, 伊吹裕子, 吉岡 寿, 五島綾子*: 静岡県立大学大学院生活健康科学研究科, *一般教育化学, 422 静岡市谷田 52-1。

ever, when another or shoulder peak is appeared adjacent to the main peak, the T_1 value obtained by inversion recovery method may be an apparent one. In this study, we changed pulse interval (PI) after 180° pulse in the time region, where magnetization changes the sign and measured the T_1 value by observing transient spectra at each PI⁹⁾.

2. Materials and Methods

2.1 Materials

Proteose peptone and yeast extract were purchased from Difco Laboratories, USA. All other chemicals used were guaranteed grade.

2.2 Cell cultivation

Tetrahymena pyriformis cells were grown in a medium containing 2% proteose peptone, 0.1% yeast extract, 0.5% D-glucose and 0.0005% $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$, pH 7.0, at 28°C for 24 h.

2.3 Irradiation of cells

Logarithmically grown culture (200 ml) was irradiated in Irradiation Exposure System (Pony Atomic Industry, PS-600SB, ^{137}Cs dose rate : 0.35 Gy/min) at 28°C for a given time. Unirradiated cells were allowed to stand at 28°C for the same period.

2.4 NMR measurement

The NMR spectra were measured with a JOEL JNM-GSX 500 spectrometer at $28.0 \pm 0.5^\circ\text{C}$. Unirradiated and irradiated cells were collected by centrifugation at 10 000 rpm ($11\,000 \times g$) at 4°C for 10 min. The medium was carefully removed from the sample of *Tetrahymena pyriformis* cells. The cells (ca. 0.3g wet weight) were gently packed into the bottom of a 5 mm glass NMR tube. NMR spectra were measured by the following double pulse sequence⁹⁾. All the magnetization is first turned over by a 180° pulse and then returned to the original equilibrium state by T_1 . The transient spectrum at PI after 180° pulse is ob-

servable by turning the magnetization with a 90° pulse. Midway in this relaxation process, the magnetization becomes very small and changes the sign around $\text{PI} = \ln 2 \times T_1$, assuming that the magnetization decreases single-exponentially. In this experiment, the measurements were carried out without any references after the resolution was adjusted by using D_2O in an another NMR tube because we focused attention on the spectral change with PI values and the insertion of a capillary tube of D_2O solution into a NMR tube resulted in lowering resolution. The measurement of T_1 value took about 20 min.

3. Results and Discussion

Figure 1 shows the NMR spectra of unirradiated *Tetrahymena* cells measured at 28°C at

Fig. 1 ^1H -NMR spectra of water in unirradiated *Tetrahymena pyriformis* cells in the relaxation state.

various PI values. The top spectrum is one at 10 s, that is, at infinite PI. As PI values decreased, the signal became small and the peak changed downward. The process of turning spectrum shape was relatively uniform, showing that the peak was composed of one component. The relative intensities of the peaks varied gradually with PI and the great change was induced at the PI which inform about T_1 . In unirradiated *Tetrahymena* cells, the PI was 1.38 s, which gave $T_1 = 1.98$ s.

Figure 2 shows the spectra of *Tetrahymena* cells irradiated about 100 Gy at 28°C. The peak B, which was hidden by the main peak A at the first stage, was observed with the decrease in PI. The peak A changed greatly with PI, but the peak B varied gradually with PI. The peak B disappeared

Fig. 2 ^1H -NMR spectra of water in γ -ray irradiated *Tetrahymena pyriformis* cells in the relaxation state.

by the addition of D_2O (data not shown). This evidence indicated that the peak B also corresponded to the protons of water, not another protons such as sugar, which usually has similar chemical shift to that of water. It was assumed that the water in irradiated cells was composed of two components, which were observed as peak A and B. Each T_1 value of peak A and B was measured using the equation $T_1 = \text{PI} / \ln 2$.

Figure 3 shows the effect of irradiation time (irradiation dose) on T_1 value of water in *Tetrahymena* cells, where unirradiated cells were allowed to stand for the same period with irradiation. The T_1 value of water in the medium obtained by the same procedure was about 2.5 s. This indicated that water mobilities in both unirradiated and irradiated cells were low compared with that of

Fig. 3 Changes of T_1 value of water in *Tetrahymena pyriformis* cells as a function of irradiation time or standing time.

□ : unirradiated cells,
○ : peak A in γ -ray irradiated cells,
● : peak B in γ -ray irradiated cells,
▲ : T_1 value in medium

Time (h) on the abscissa indicates an irradiation time in the case of irradiated cells and a period left on standing in the case of unirradiated cells.

bulk water. The spectra after 1 h irradiation were nearly similar to those in unirradiated cells. The T_1 values of main peak A in irradiated cells tended to be larger than those in unirradiated cells. This phenomenon was similar to that in tumor cells.

A new peak B appeared after the irradiation for 3 h. All of T_1 values of peak B in irradiated cells tended to be small compared to those in unirradiated cells, indicating that the physico-chemical property of water of peak B was different from that of peak A. Even if there are two kinds of water in cells, the ^1H -NMR spectrum of water is usually observed as a single peak by their rapid exchanges^{6,7)}. Therefore, peak B suggests the appearance of a different kind of water in the cells by the irradiation. It was reported that the autophagic vacuoles were increased in human urinary bladder cells by the X-ray irradiation⁸⁾. The peak B might reflect the appearance of vacuoles induced by γ -ray irradiation in *Tetrahymena pyriformis*. The decrease of T_1 values for peak B suggests the increase of the structured water like that found in developing muscle.

The T_1 values of the peaks in unirradiated *Tetrahymena* cells and in irradiated cells tended to decline with time. The standing of cells without stirring lead to the oxygen-deficiency in cells and such stresses may bring about the increase in bound water. But it has not been cleared yet.

In conclusion, NMR method revealed that γ -ray irradiation of *Tetrahymena* cells resulted in the appearance of another water different from the ordinary water found in unirradiated cells.

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