Uptake and Release of Some Radionuclides by Fresh Water Phytoplankton in Batch Culture

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The uptake and release of radionuclides, $^{137}$Cs, $^{65}$Zn, $^{60}$Co and $^{45}$Ca, by fresh water phytoplankton Anabaena variabilis, which is one of the bluegreen algae, were followed through all growth phases of algae. The amount of adsorption of radioactivity on cellular surface of algae fixed with formarin solution was smaller than that radioactivity taken up by living algae. The radionuclides, except for $^{45}$Ca whose stable element was one of the elementary nutrients, were noticeably concentrated on the early phase, especially on the exponential growth phase. Thereafter, the activity concentration in algae were decreased by biological dilution due to cell division and reached the equilibrium. The degree of the decrease of radioactivity in algae due to biological dilution was in the order, $^{60}$Co, $^{65}$Zn, $^{137}$Cs. In the case of $^{45}$Ca, the phenomenon of biological dilution was not observed on every growth phase. The uptake and release of radioactivity by algae occurred in the dark, but the amount of radioactivity taken up was smaller than in the light.

INTRODUCTION

A transfer and a redistribution of radionuclides released to an aquatic environment depend on a geochemical behavior and a biological transport of the radionuclides. Reproductivity of organisms is based on the productivity of algae, and phytoplanktons seem to occupy an important ecological niche as a primary producer and as a circulator of radioactive materials in an aquatic ecosystem.

Most of the investigations in the past treated of the effects of light, pH, temperature and co-existing ion concentration on the accumulation of radionuclides by phytoplankton. There were few studies about effects of biomass of algae or of the division rate of the algal cell on the uptake of radionuclides. For the former problem, there was a discrepancy in opinions between Polikarpov and Fedrov. For the latter, Rice et al., Watt et al. and Honda et al. showed experimentally that the radioactivity concentration in algae was decreased by biological dilution due to cell division of algae. The effects of biomass and biological dilution due to cell division on the concentration of radionuclides in algae should be unificatively considered.

In this paper, the experiments were performed to study the accumulation process...
of radionuclides on the three growth phases by uni-cellular phytoplankton.

MATERIALS AND METHODS

Radionuclides

Radionuclides used in the experiment were chlorides of $^{137}$Cs, $^{65}$Zn, $^{60}$Co and $^{45}$Ca, which were supplied on authorization from the Isotope Association of Japan. Experiments were performed without carriers except for $^{45}$Ca. Stable calcium compound was added as a nutrient to the medium shown in Table 1.

Phytoplankton

A kind of blue-green algae, Anabaena variabilis, was used in all experiments. This phytoplankton is a uni-cellular alga which lives in fresh water. The algal cell divides into two autospores. The diameter of the alga is about a few micrometers.

Algal culture

The culture medium of 300 ml sterilized was poured into the flat culture bottle of a capacity 500 ml and algae, Anabaena variabilis, were inoculated into the medium. They were in pure culture, and thus were sufficiently free from bacteria to make their use valid. Apparatus for the culture and the culture medium were sterilized with an autoclave at the temperature 120°C and at the pressure 2 atmos. Constituents of the medium prepared for the experiments were shown in Table 1. It was a modified medium based on the Kratz-Myers'. The bottle was set in an aquarium filled with water in constant illumination with daylight fluorescent lamps and at a temperature of 30±2°C during the experiments. The medium was continuously aerated with air including 5% CO$_2$ gas. This mixed gas was sterilized through 0.1% solution of corrosive sublimes.

Measurement of algal density

Algal density was determined by the measurement of turbidity. A nephelometer ana-14S (Tokyo Photo-Electric Co., LTD) was used to measure the turbidity. The algae were collected on a membrane filter (AAWP Millipore, 0.8 μm), dried in a desiccati for 3 hours at room temperature and weighed. Thus it was possible to develop a relation between algal density and optical density, and a linear relationship with a

| Constituents of Culture Medium | KNO$_3$ : 3.0 g | A$_5$ Solution | H$_3$BO$_3$ : 2.86 g |
|-------------------------------|----------------|--------------|---------------------|
| MgSO$_4$·7H$_2$O              | 0.25 g         | MnCl$_2$·4H$_2$O : 1.81 g |
| CaCl$_2$·2H$_2$O              | 0.025 g        | ZnSO$_4$·7H$_2$O : 0.22 g |
| K$_2$HPO$_4$                  | 1.0 g          | CuSO$_4$·5H$_2$O : 0.08 g |
| Fe$_2$(SO$_4$)$_3$·6H$_2$O    | 0.004 g        | Na$_2$MnO$_4$ : 0.021 g |
| C$_6$H$_{12}$O$_7$Na$_3$·2H$_2$O | 0.165 g | Conc. H$_2$SO$_4$ : 1 drop |
| A$_5$ Solution                | 1.0 ml         | Distilled water : 1.0 |
Coefficient of correlation of 0.994 was obtained between them.

Measurement of radioactivity and its correction

The algae were collected on a membrane filter (AAWP) from the culture bottle and transferred into a sample dish. The radioactivity in all samples was detected by a low-background gas flow counter. The radioactivity in the medium was also measured.

In order to correct the counted radioactivity taken up by algae for the radioactivity adsorbed on the membrane filter, the culture medium including radioactivity was filtered and the radioactivity retained on the filter was counted. Figures 1 and 2 show the relation between the effluent of the medium filtered and the adsorption amount of radioactivity on the membrane filter. As a linear relation was obtained except for $^{45}$Ca, the amount of adsorption was calculated according to the following equation,

\[ A = k \cdot x \]  

where \( A \) is the amount of radioactivity adsorbed on the filter (cpm), \( k \) a proportional constant (cpm/ml) and \( x \) the effluent of the medium (ml). A value of \( k \) was determined for every experiment according to the activity concentration in the medium. In the case of $^{45}$Ca, since the amount of radioactivity adsorbed was not changed for the effluent more than 5 ml of the medium, the corrected amount was taken as a constant independently of the effluent. Radioactivity retained on the filter was very low.
as comparing with the radioactivity in the medium filtered for every radionuclide.

The correction was made for the self adsorption of $\beta$-ray by algae. As the energy of $\beta$-ray has a continuous spectrum, it is not so simple as $\gamma$-ray to correct the self-adsorption of $\beta$-ray. However, to simplify the correction, the adsorption equation of $\beta$-ray was taken as follows,

$$1 = I_0 e^{-\mu X_0}$$

(2)

where $I_0$ is the intensity of $\beta$-ray before incidence into absorber (cpm), $I$ the intensity of $\beta$-ray after passing through the absorber (cpm), $X_0$ the thickness of the absorber (mg/cm$^2$) and $\mu$ the mass absorption coefficient (cm$^2$/mg). Considering the self-absorption by algae, the following equation can be obtained,

$$N = \int_0^{X_0} N_0 e^{-\mu x} dx = \frac{N_0}{\mu} (1 - e^{-\mu X_0})$$

(3)
where $x$ is the thickness of algae (mg/cm²), $N_0$ real activity in algae per unit thickness (cpm/(mg/cm²)), and $N$ apparent activity (cpm). Experimental results on the self-absorption of $\beta$-ray are shown in Figures 3 and 4. Taking account of both corrections mentioned above, the following equation can be obtained as a whole,

$$N = \frac{N_0}{\mu}(1-e^{-\mu x}) + A \cdot e^{-\mu x_0}$$

(4)

after all, the real radioactivity in algae per unit weight is shown as follows,

$$N_0 = \frac{\mu(N - A \cdot e^{-\mu x_0})}{1-e^{-\mu x_0}}.$$  

(5)

Mass absorption coefficients of algae obtained from the experiments and values of the maximum energy for each radionuclide are shown in Table 2.

| Nuclide | Mass Absorption Coefficient | Maximum Energy of $\beta$-ray |
|---------|-----------------------------|-----------------------------|
| Ca-45   | 0.11 cm²/mg                 | 0.254 Mev                   |
| Co-60   | 0.075                       | 0.312                       |
| Zn-65   | 0.050                       | 0.324                       |
| Ca-137  | 0.025                       | 0.514                       |

**Adsorption of radionuclides on cell surface of algae**

Algae cultured in non-active medium on the stationary phase were soaked in 10% formalin solution so as to fix them. They were collected on the membrane filter, washed with distilled water and then transferred to the new medium including radionuclide. Air with CO₂ gas was blown into the bottle to mix the medium. We periodically sampled algae out of the medium and counted the radioactivity adsorbed on
the cell surface of algae. The measured value was corrected by the method mentioned above.

Growth process of algae and concentration process of radionuclides

Algal growth in cultures of limited volume can be generally divided into three growth phases, that is, (1) logarithmic phase, (2) linear phase, (3) stationary phase. Here, uptake and accumulation of radionuclides by algae on each growth phase were followed.

In the experiment on the logarithmic phase, a small amount of algae was inoculated into the new medium and after domestication for a few hours, radionuclide was added to the medium. Algal density, and radioactivities in algae and in the medium filtered were periodically determined.

In the experiment on the linear and stationary phases, after the algae were inoculated and left as it was until they grew at each phase, the radionuclides were added and the experiments were started as the same way above.

Excretion of radionuclides and biological dilution by cell division

Algae were cultured in the light and in the dark, and the mode of the decrease of the activity concentration in algae was compared for the both cultures in order to examine biological dilution of the activity concentration in algae due to division of cells and physiological excretion of radionuclides from algal cells.

Excretion of radionuclides and biological dilution by cell division

RESULTS AND DISCUSSION

Adsorption of radionuclides on algal surface

Radionuclides of $^{137}$Cs and $^{65}$Zn adsorbed on cell surface of algae could not be significantly detected in six hours after the experiments were started. As the adsorption of radionuclides on the algal surface seems to cease relatively in a short time, this experiment were continued for as long as only six hours. It is considered that the transport by diffusion of ions through a membrane of algae will be dominant in a long time. The experimental results of $^{60}$Co and $^{44}$Ca were shown in Figure 5. These radionuclides were adsorbed in the first two hours and then the amount of adsorption was gradually increased. Since algae were fixed with formalin solution in this experiment, the state of cell surface might be different from the normal surface of living cell. It will be difficult to distinguish experimentally the radioactivity adsorbed on the cell surface of fixed algae from the radioactivity taken up into the cell of living algae.

Uptake and release of radionuclide by algae

Experimental results of $^{137}$Cs are shown in Figures 6-10. Activity concentration
in algae reached equilibrium in about 50 to 100 hours and the concentration factor of $^{137}$Cs by *Anabaena variabilis* ranged from 25 to 30.

Figure 6 shows the changes of the activity concentration in algae in the experiments performed through the whole growth phases. Radionuclide was rapidly taken up by algae on the logarithmic growth phase and the decrease of the relative concentration in algae due to the biological dilution was found on the linear growth phase. Here, the relative concentration means the ratio of the activity concentration in algae to that in the culture medium at the time when algae were sampled on the concentration process. Figures 7 and 8 are the results performed on the linear and stationary phases. In these cases, the decrease of the relative concentration was not
observed and the relative concentration increased monotonously. In Figure 7, uptake of radioactivity by algae with the rapid growth rate (Run I) was compared with that by algae with the slow growth rate (Run II). The faster the growth rate of algae is, the earlier the time reaching the equilibrium is. The concentration factor, which is the relative concentration at equilibrium, was however not different for both cases. Figure 8 shows the result of the experiment made under the condition of illumination. Comparing with the experimental result in the dark shown in Figure 9, although the algal density is the same for both runs, the relative concentration in algae in the dark was lower than in the light because the cells lose vigour.
Run I and Run II in Figure 10 show the changes of the activity concentration in algae grown in the light and in the dark respectively. The activity concentration in algae grown in the light decreased rapidly with the growth of algae. The activity concentration in water in the light was negligibly low, so it was not shown in the figure. Algal density was constant in the dark and the small decrease of the activity concentration in algae due to the physiological excretion from cells was observed. The decreasing rate of the concentration in algae was not remarkable comparing with that in algae grown in the light. In this case, the excreted radioactivity resulted in raising the activity concentration in the medium; therewith the activity concentration in algae was raised again. It seems that uptake and excretion of $^{137}$Cs by *Anabaena variabilis* occurred under the condition of the culture in the dark in the same way as
in the light even if the physiological function of algae would be lowered.

Figure 11 shows the experimental results of $^{65}$Zn uptake. The experiment of Run I in Figure 11 was started from the logarithmic growth phase and that of Run II was started from the linear growth phase. Early concentration of radioactivity in algae were high for both cases and the concentration decreased with the growth of algae. Comparing with the results of $^{137}$Cs, the decreasing rate of the activity concentration of $^{65}$Zn in algae was faster than that of $^{137}$Cs, and differing from $^{137}$Cs, the decrease of the concentration was found on the linear phase. In order to confirm this phenomenon, the same experiments were repeated several times but the same modes of the concentration of $^{65}$Zn were observed. Although not shown in Figures, the relative concentration of $^{65}$Zn in algae increased monotonously on the stationary phase similarly to the uptake of $^{137}$Cs shown in Figure 8. Figures 12 and 13 show the experimental results on the excreation of $^{65}$Zn from the cells in the light and in the
dark respectively. The decrease of the activity concentration of $^{65}$Zn in algae in the light was noticeable due to biological dilution comparing with in the dark.

Figure 14 shows one of the experimental results of $^{60}$Co uptake. The activity concentration of $^{60}$Co in algae attained at the peak concentration after 10 hours and decreased faster than $^{65}$Zn and $^{137}$Cs.

Figure 15 shows the uptake mode of $^{45}$Ca. Differing from the other nuclides, the activity concentration of $^{45}$Ca in algae did not decrease on any of growth phases. Stable calcium compound was added to the medium as a nutrient in this experiment. Algae may take up both of stable and radioactive calcium according to their growth.
CONCLUSION

The self-absorption of $\beta$-ray must be corrected when $\beta$-activity in algae collected on a membrane filter are determined by a gas flow counter. The real activity can be calculated by using the equation (5).

The accumulation of radioactive nuclides by algae may be caused by physical adsorption on cell surface, diffusion, and active transport through cell membrane. Some radionuclides are taken up and excreted at a different rate on each growth phase. Others are taken up rapidly on the early time and stored in the cell, thereafter are little taken up even if algae would be grown. The latter case has been reported to be noticed for some nutrients.\textsuperscript{11,18}

It was observed that the uptake and release of $^{137}$Cs by algae occurred also in the dark though slow. The uptake mode of $^{137}$Cs, $^{65}$Zn, and $^{60}$Co in the light were similar. Their radionuclides were markedly concentrated by Anabaena variabilis on an exponential growth phase, and the peak concentration was observed at the time when the growth phase reached a linear one; thereafter the activity concentration in algae decreased with time due to biological dilution. While $^{45}$Ca concentration in algae increased monotonously on every growth phase. Therefore, when the relation between biomass of algae and the concentration factor of radionuclide, on which opinion is divided, is discussed, it is necessary to take account of the concentration mechanisms of radionuclides and biological dilution of the activity concentration in algae by division of cells. The concentration factor for algae at a section on the growth process of algae is a problem taken part in the rate of cell division which occurred in the individual level of an algal cell and it is not essential for interpretation of the relation between them.

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