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Effect of selenium on arsenic metabolism in *Cylindrotheca fusiformis*

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Effects of selenium (Se) on the kinetics of incorporation and excretion of ^{74}As have been studied by use of a marine diatom, *Cylindrotheca fusiformis*. For the incorporation experiment, the cells were incubated with carrier-free ^{74}As in a phosphate-free normal medium, and collected sequentially over 45 h. For the excretion experiment, the cells were transferred to a normal medium and collected sequentially over 95 h. The cell components were fractionated into water-soluble, insoluble and lipid fractions by TLC. ^{74}As was mostly incorporated into the water-soluble fraction and less than 10% of it into the insoluble and lipid fractions. Within a few hours considerable amounts of ^{74}As were found in those fractions, but after that the rate of accumulation decreased. ^{74}As was excreted into the medium rapidly at the beginning, but the rate of excretion slowed down gradually. In the cells, the amount of As in the insoluble fraction decreased more rapidly than that in the lipid and water-soluble fractions. The time course changes of ^{74}As in the three fractions showed some oscillation of larger amplitude of variation at first and later approached a constant value. Selenium in the medium showed some effects on the rate of arsenic incorporation as well as on the ratio of the three fractions. In the excretion process, selenium changed the amplitude of the variation and the ratio of ^{74}As in the three fractions at the later times.

Keywords: Arsenic metabolism, selenium, arsenate, selenate, *Cylindrotheca fusiformis*, marine diatom, lipid fraction, soluble fraction, insoluble fraction, ^{74}As

INTRODUCTION

Selenium is an element which has effects on the physiological behavior of arsenic (As) in animal cells,[1,2] but these relations seem to vary according to the physiological conditions.

To regulate the metabolism of arsenic in living cells, the rigid rules governing it should be understood. The effects of selenium on this system may suggest some clues as to further understanding of the interactions with arsenicals.

The authors wished to enquire whether and/or how selenium affects arsenic metabolism in a marine alga, *Cylindrotheca fusiformis*.

EXPERIMENTAL

Culture

Cylindrotheca fusiformis was grown in a normal medium (see under Culture medium, below) for several weeks until the culture reached 1.8×10^6 cells per cm^3, and was then transferred to a new medium without phosphate salts. After one week the cells were collected, washed thoroughly with a fresh normal medium without phosphate and mixed with a ^{74}As medium with or without selenate solution.

The culture was carried out under 2700 lux from fluorescent lamps, at 26°C, on a rotary shaker.

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Culture medium

For the incorporation experiment, a medium was prepared from the normal medium but without phosphate.

For the excretion experiment, the normal medium (GPM medium[3,4], modified) was used, i.e. artificial seawater[a] was mixed with K_2HPO_4 (2 mM) and potassium nitrate (KNO_3, 0.2 mM), with minor elements[b] and vitamins.[3]

Sampling

Using a device to isolate the cells from the medium under sterile conditions,[5] the cell suspensions were collected at the time intervals indicated in the figures. Cells were collected by centrifugation and washed thoroughly with sterilized artificial seawater. A portion of the cells was stored below −20°C for analysis.

Radioactive arsenic

^{74}As-arsenate (carrier-free in 0.04 M HCl, 63 MBq cm^{-3}) solution was added to the medium followed by the addition of an equivalent amount of potassium hydroxide (KOH) solution.

Selenium concentration

Sodium selenate solution (2.4×10^{-7} M and 2.4×10^{-5} M for incorporation experiments; 1×10^{-6} M and 1×10^{-4} M for excretion experiments) was mixed with the culture medium for the selenium addition experiment.

Determination of chlorophyll content

A portion of the cells was extracted with 80% acetone and the optical density (OD) measured at 663 nm and 645 nm using a Beckman/Bausch & Lobm spectrophotometer, and the chlorophyll contents were calculated.[6]

Fractionation of the cell components

Arsenic compounds in the cells were fractionated into water-soluble fraction, lipid fraction and insoluble fraction by thin layer chromatography (TLC).

Thin-layer chromatography

The cells suspended in a small volume of water were repeatedly frozen and thawed and then spotted at the origin of a cellulose-powder TLC plastic plate. The thin-layer plates were developed in ascending manner with phenol saturated with water. After rechromatography with water,

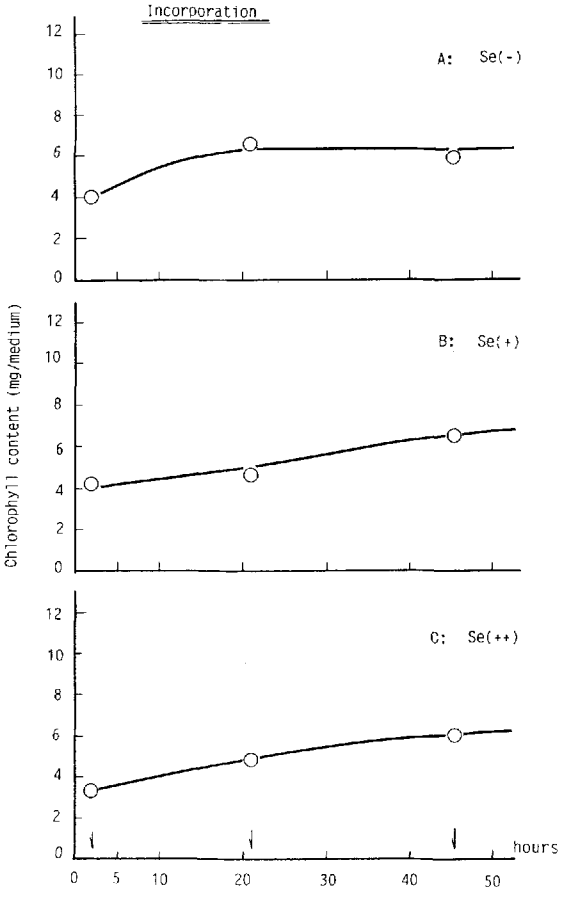

Figure 1 Time course changes of chlorophyll contents in the incorporation process.

In the ^{74}As-incorporation experiment, the medium was devoid of phosphate. A portion of cultured cells were collected, and chlorophyll was extracted with 80% acetone. From the optical density at 645 nm and 663 nm, the chlorophyll contents were calculated.[6] A, the cells were cultured in a medium with selenium absent; B, the cells were cultured in a medium containing 2.4×10^{-7} M selenate; C, the cells were cultured in a medium containing 2.4×10^{-5} M selenate.

a. The composition of the artificial seawater in g/l:NaCl, 28.32; KCl, 0.77; $MgCl_2 \cdot 6H_2O$, 5.41; $MgSo_4 \cdot 7H_2O$, 7.13; $CaCl_2 \cdot 2H_2O$, 1.56; $NaHCO_3$, 0.20.

b. The composition of minor elements in mg/l medium: Na_2EDTA, 30; $FeCl_3 \cdot 6H_2O$, 1.45; H_3BO_3, 34.2; $ZnCl_2$, 0.3; $MnCl_2 \cdot 4H_2O$, 4.3; $CaCl_2 \cdot 6H_2O$, 0.13.

the chromatograms were dried and cut into insoluble fractions, water-soluble fractions and lipid fractions. The respective fractions were measured in a scintillation liquid solution.[7]

Determination of radioactivity

A small portion of the samples was measured in a vial using scintillation liquid (toluene/PPO[c]/POPOP[c], 1 litre:3.92 g:80 mg) using a Beckmann-liquid scintillation counter. The radioactivity on the thin-layer plates was determined by immersing small pieces of these plates in the liquid scintillation solution.

c. Abbreviation used: PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2(5-phenyloxazoly)] benzene.

Reagents

Reagents were Analytical Reagent grade or Guaranteed Reagent (JIS) grade.

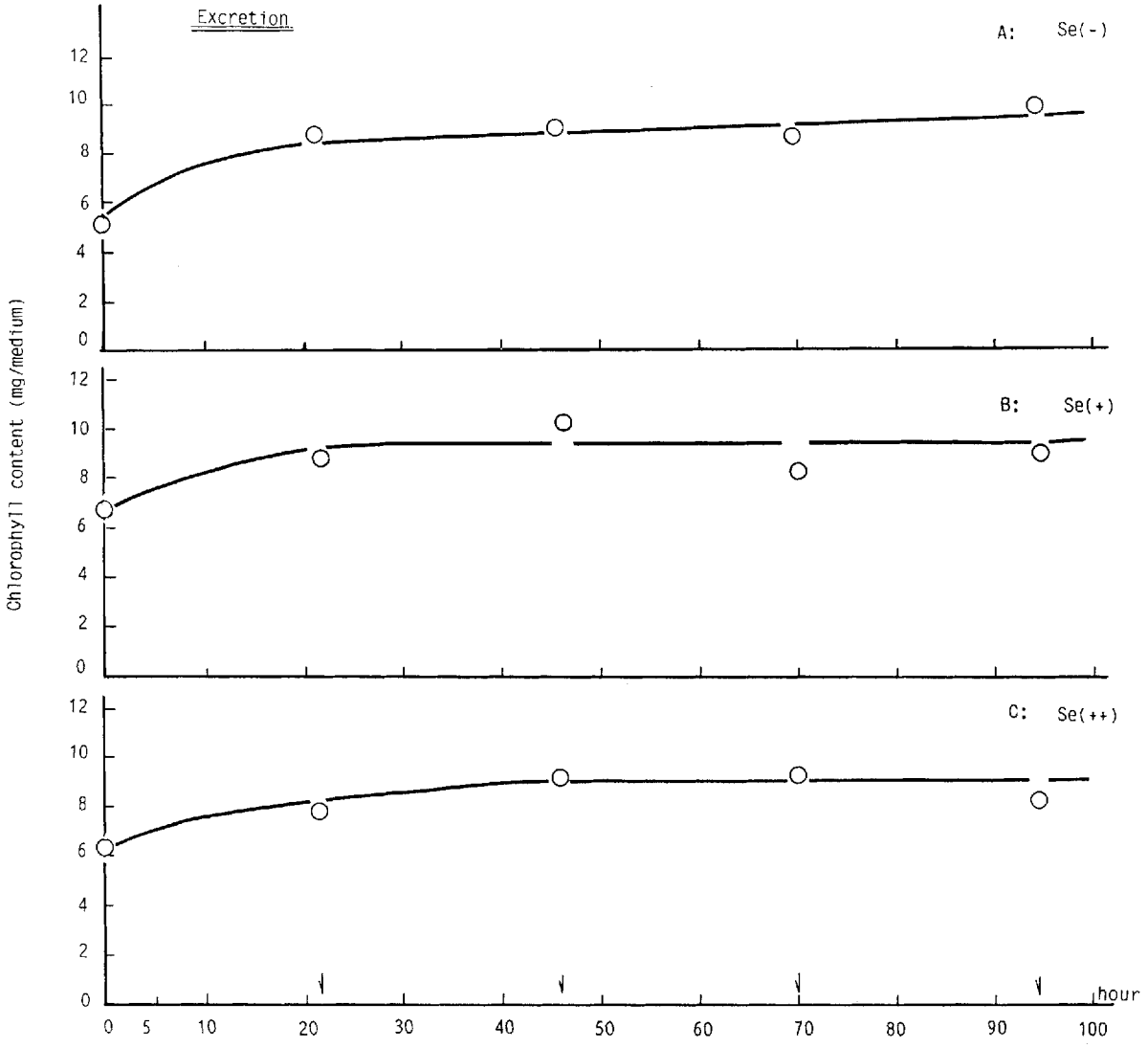

Figure 2 Time course changes of chlorophyll content in the excretion process. After the incorporation experiment, the cells were transferred into a complete medium and collected at time intervals of 0 h, 21.5 h, 46 h, 70 h and 94.5 h. The chlorophyll content was measured as described in Fig. 1. The cells were cultured in a medium (A) with selenium absent (B) containing 1×10^{-6} M selenate; (C) containing 1×10^{-4} M selenate.

RESULTS

Time course changes of chlorophyll contents

During the incorporation experiment, the cells were monitored by their chlorophyll content (Fig.1). The increment of the chlorophyll content showed a reduction in the cells cultured with a selenium increment.

During the excretion experiment, the chlorophyll contents were determined as shown in Fig. 2. Their time course changes after 50 h were not very different in the cells cultured either with or without selenium in the medium, although a trend was observed in that the increment of chlorophyll content was slightly slower for the cells in the selenium medium.

Incorporation of ^{74}As into the cells

When the 'carrier-free' (low-level carrier) [^{74}As]arsenate was added to the culture medium, the radioactivity was mostly observed in the water-soluble fractions within 1 h (Fig. 3). This incorporation was very rapid, as if it was due merely to adsorption on the surface of the cells, but the rapid appearance of radioarsenic in the

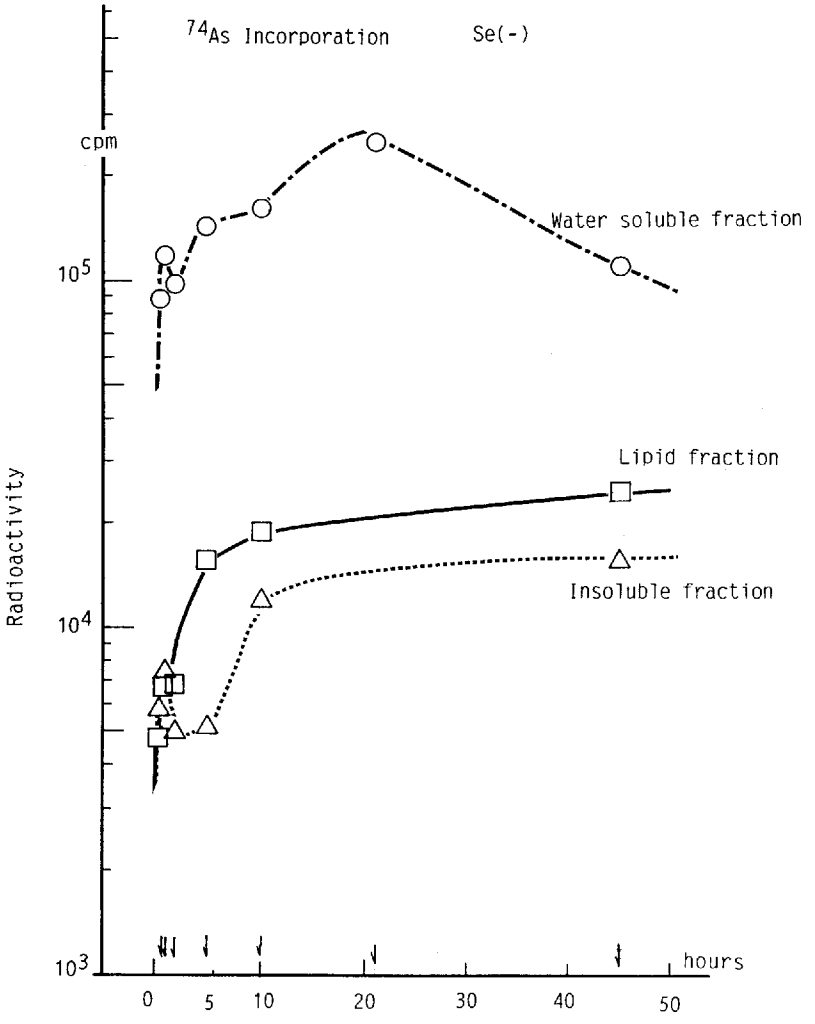

Figure 3 Time course changes of ^{74}As in water-soluble fraction, insoluble fraction and lipid fraction in the ^{74}As-incorporation process, without selenium. The cells were cultured in a phosphate-deficient medium without selenium. After the addition of ^{74}As-arsenate, the cells were collected at 30 min, 1 h, 2 h, 5 h, 10 h, 21 h and 45.5 h, washed thoroughly with the cold washing medium and separated into the three fractions by TLC.

insoluble and lipid fractions indicated that some arsenic was incorporated into the cells very rapidly. As the collected cells were washed thoroughly with fresh artifical saline solution, the water-soluble arsenic fraction must arise from that inside the cells. The incorporation rate of ^{74}As was greatest into the lipid fraction and slower into the insoluble fraction. The latter contains protein-bound arsenic as well.[8]

The doubling time of ^{74}As incorporated into the lipid fraction was about 3 h initially and that into the water-soluble fraction was 5–10 h at the outset. The total ^{74}As in the water-soluble fraction exceeded that in the lipid fraction by a factor of 10. Within 20 h after the incubation, the incorporation rate into them slowed down, suggesting that these reactions changed to other routes.

Effect of selenium addition on the incorporation of ^{74}As into the cells

The incorporation rate of the ^{74}As appeared to be affected by the addition of selenate (2.4×10^{-7} M) in the medium (Fig. 4). The effect was mostly found in the incorporation of ^{74}As into the insoluble fraction, as the incorporation continued longer than that in the normal cells in a non-selenium medium, as in Fig. 3. The ^{74}As incorporation into the lipid fraction by cells with

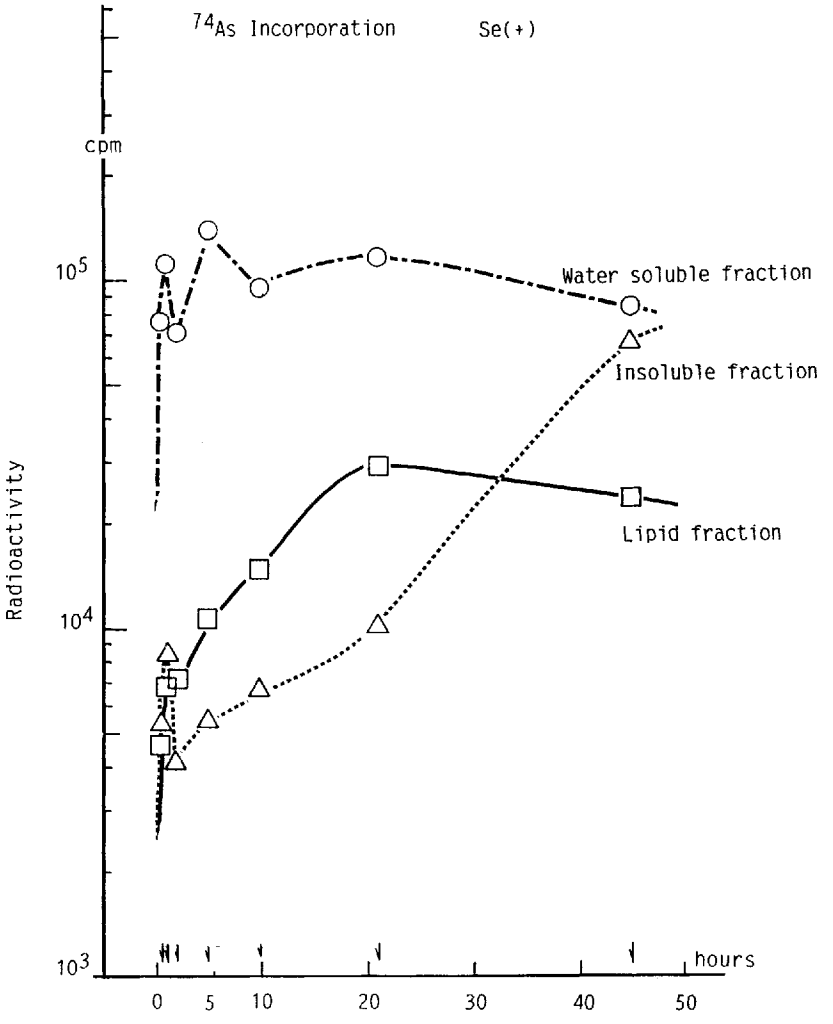

Figure 4 Time course changes of ^{74}As in the water-soluble fraction, insoluble fraction and lipid fraction, in the ^{74}As-incorporation process, with 2.4×10^{-7} M selenate. The cells were sampled and treated as described in Fig. 3.

selenium took a longer time to reach a plateau than that by the cells without selenium. The incorporation of ^{74}As was largely into the water-soluble fraction, but it reached a maximum more quickly than that in selenium-free cells (Fig. 3).

Effect of a high concentration of selenium

At the higher selenium concentration, ^{74}As incorporation was initially rapid but then slowed (Fig. 5). Incorporation into the lipid fraction was very rapid at first but decreased rapidly, as if incorporation had ceased, apparently changing to another mechanism.

Excretion process of ^{74}As from the cells

By changing the culture medium to a fresh normal medium containing phosphate, the cells excreted ^{74}As into the medium, producing a decrease in the amount of ^{74}As in the cells. The cells cultured without selenate were transferred to a normal medium and behaved as shown in Fig. 6.

The largest amount of ^{74}As accumulated in the water-soluble fraction and decreased with a half-life of 120 h. The lipid fraction decreased at the beginning, but arrived at a plateau in 20 h, showing overall a half-life of about 2 h. On the other hand the ^{74}As in the insoluble fraction fluctuated rapidly and decreased. It showed a large decrement for several hours; then the insoluble fraction

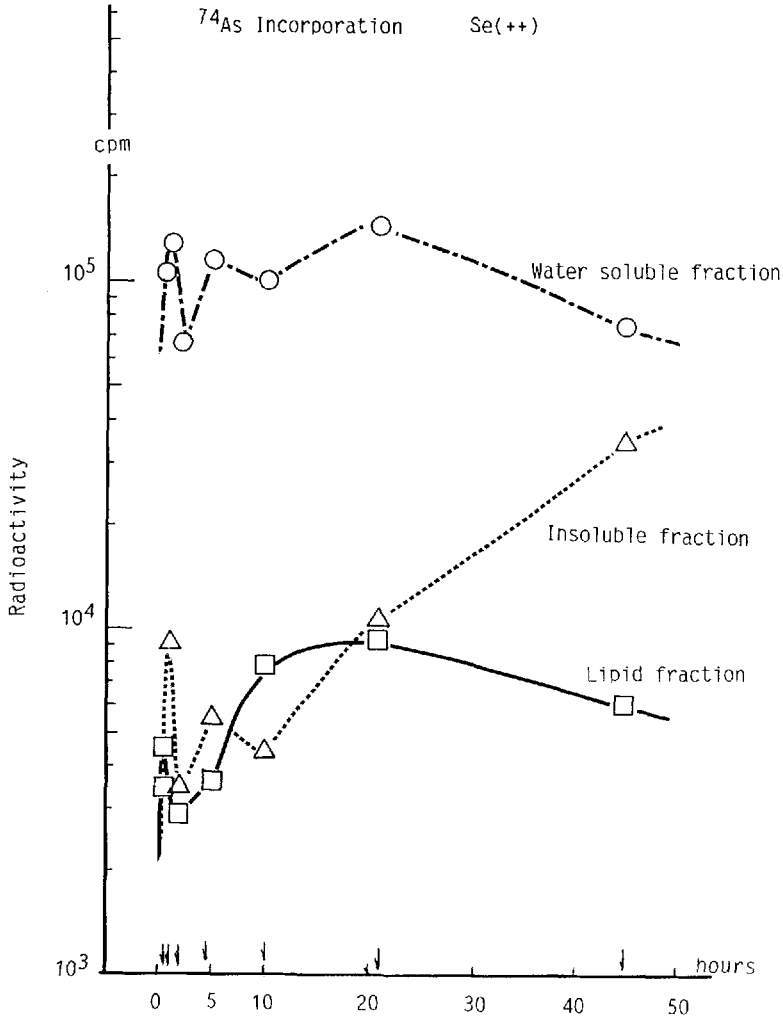

Figure 5 Time course changes of ^{74}As in the water-soluble fraction, insoluble fraction and lipid fraction in the ^{74}As-incorporation process, with 2.4×10^{-5} M selenate. The cells were treated as described in Fig. 3.

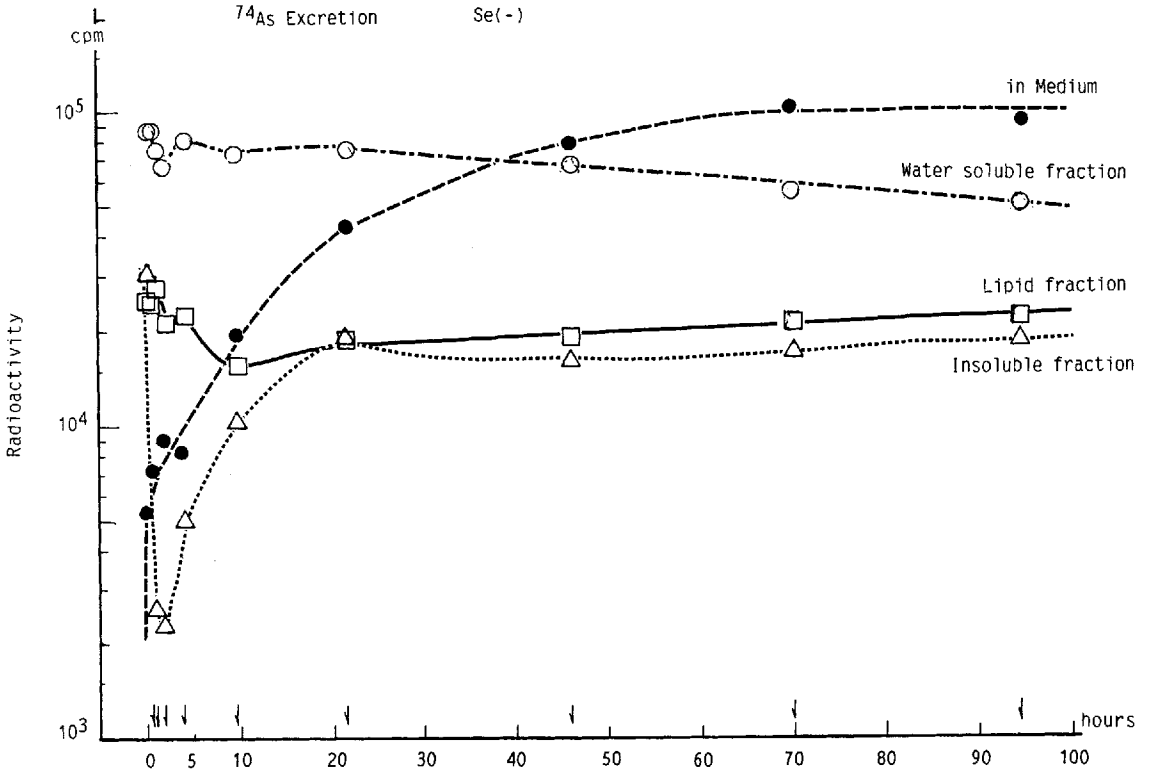

Figure 6 Time course changes of ^{74}As in the water-soluble fraction, insoluble fraction and lipid fraction in the ^{74}As-excretion process without selenium. The cells were collected after the addition of ^{74}As at 30 min, 1 h, 2 h, 4 h, 9.5 h, 21.5 h, 46 h, 70 h and 94.5 h, washed thoroughly with cold medium and separated into the three fractions by TLC.

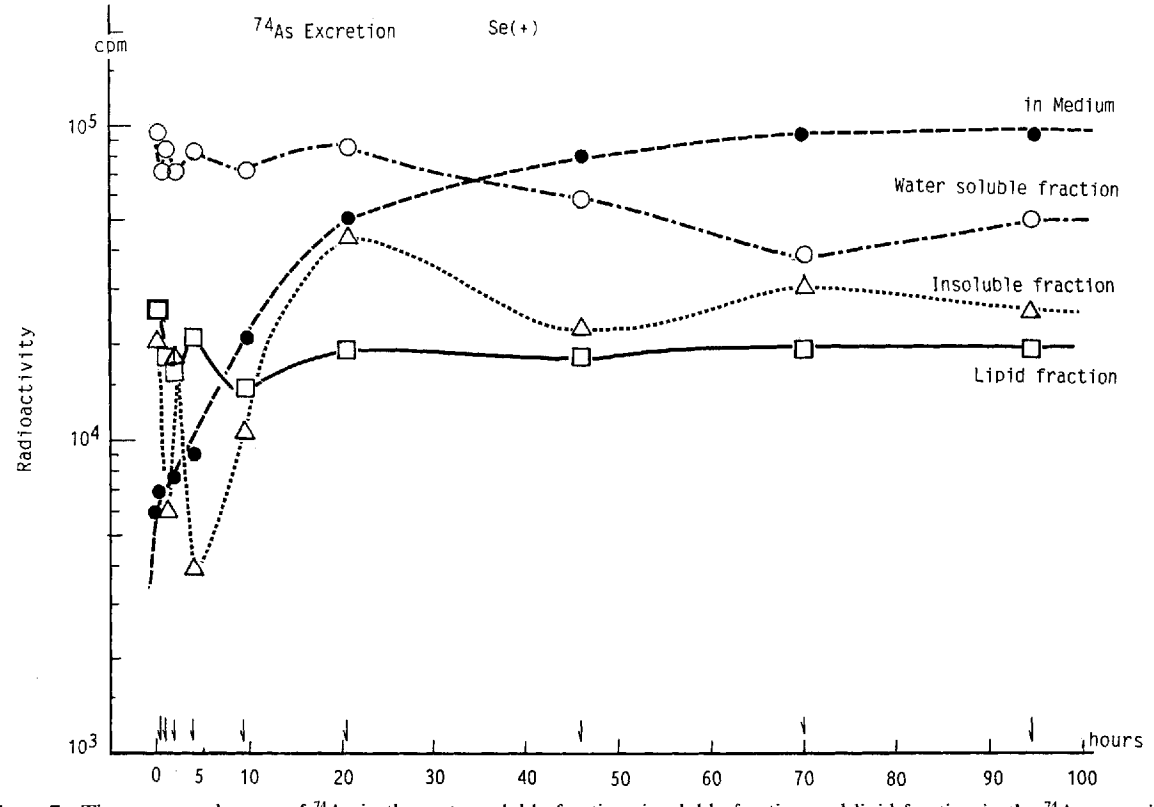

Figure 7 Time course changes of ^{74}As in the water-soluble fraction, insoluble fraction and lipid fraction in the ^{74}As-excretion process, with 1×10^{-6} M selenate. The cells were treated as described in Fig. 6.

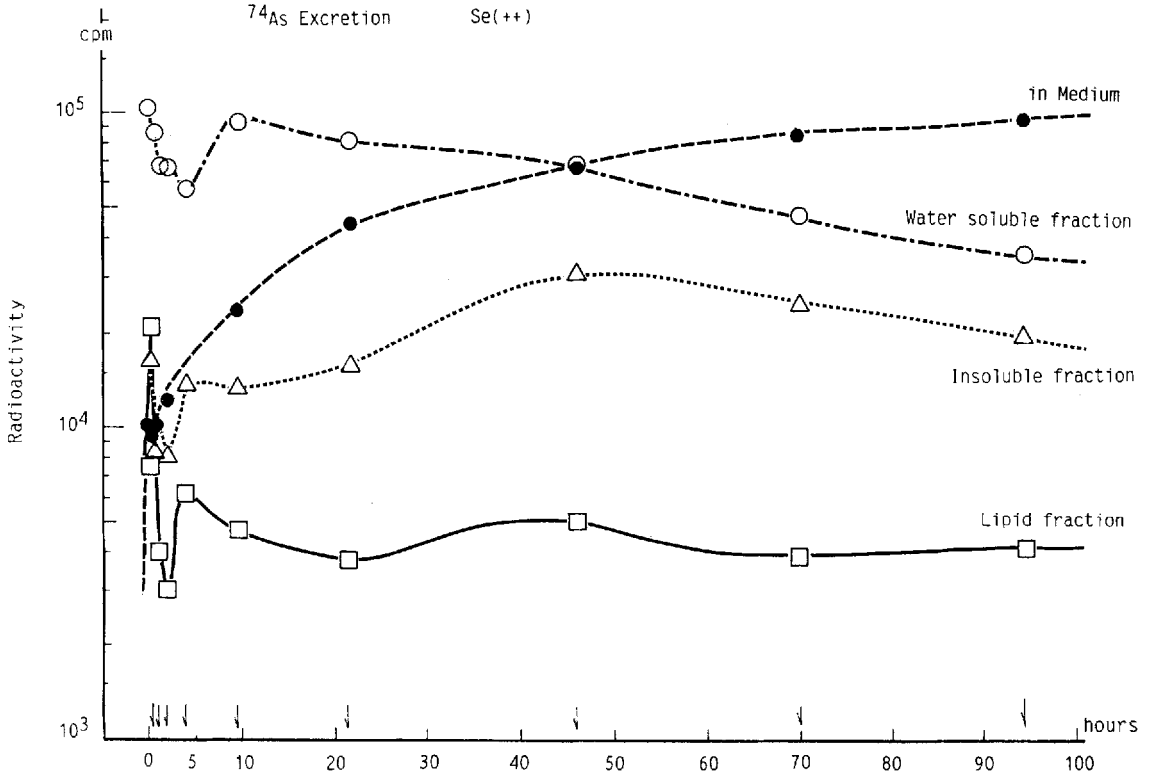

Figure 8 Time course changes of ^{74}As in the water-soluble fraction, insoluble fraction and lipid fraction in the ^{74}As-excretion process, with 1×10^{-4} M selenate. The cells were treated as described in Fig. 6.

increased. This phenomenon was also observed in Figs. 7 and 8.

Effect of selenium on the excretion of ^{74}As.

The time course changes of ^{74}As in the water-soluble fraction showed a more rapid and greater decrease initially in the cells cultured in the selenium-added medium than that in the cells cultured in the normal medium (Figs. 7 and 8).

The amount of ^{74}As in the lipid fraction showed a smaller variation at the beginning than the other fractions. Selenium addition appeared to make the time course changes more variable.

The insoluble fraction had an unusual pattern at first for a few hours. After that, the level of ^{74}As slowed down (Figs. 7, 8).

DISCUSSION

Selenium interferes with arsenic metabolism in animals.[1,2] This leads to a suggestion that selenium has a capability for affecting the enzyme systems of arsenic metabolism in marine algae as well. The level of selenium in the ocean is about 5×10^{-8} M in seawater, compared with 4×10^{-8} M for arsenate and 3×10^{-6} M to 3×10^{-8} M for phosphate.

This means that diatoms in the ocean live in a medium containing comparable selenate and arsenate concentrations.

The considerable incorporation of ^{74}As into the insoluble fraction within a short time initially might appear to be a temporary phenomenon observed by changing to phosphate-free medium. However, as the cells had been grown in the same medium for several days before the addition of the ^{74}As, these phenomena may also be explained by other causes. The initial rapid binding of ^{74}As by the cells in the incorporation process suggests that the arsenic receptor or the transport sites have an irreversible affinity for the arsenic, and this affinity may become sensitive during a longer culture.

We have examined the effects of higher selenium and arsenic levels on algae in order to understand the phenomena at natural concentra-

tions. The selenium level in this paper is higher than the level in the oceans, and the ratio of the selenium level to the arsenic level in this paper is higher than that of the oceans.[9] Thus, the ratio of the selenium level to the arsenic level was also examined. These concentration differences may underlie the observed fluctuation in the ^{74}As level of the insoluble fraction (Figs. 6, 7 and 8).

As several of the minor constitutents of seawater may interact[10] with coexisting metabolic systems, it is important, especially in the case of selenium and arsenic, to understand these interactions and their effects on growth and productivity.

CONCLUSION

Cylindrotheca fusiformis cultured in a medium with selenium showed a different kinetic pattern of ^{74}As-excretion as well as of ^{74}As-incorporation from that cultured in a medium without selenium. These results suggest that the coexisting selenium is important in arsenic metabolism in the ocean.

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