Participation of functional mineral-encaged zeolite treated water species in restoration of cell motility of organotin-intoxicated *Euglena gracilis* Z

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Summary

Series of research in our laboratory has shown that restoration of morphology and motility of TBTCl-intoxicated *Euglena gracilis* Z should be resulting from trace minerals in the processed aqueous solution. To examine the detoxication effect of mineral-encaged zeolite on the impaired cells, we used the bioassay system using *Euglena gracilis* Z as the model organism and TBTCl as the model xenobiotic. Under the polluted environment, *Euglena* cells changes their shape to spherical form from spindle form. By taking advantage of this unique character, we examined the different types of zeolites which encaged different minerals. TBTCl-intoxicated *Euglena* cells were separately washed with the water species processed by zeolite, then incubated in the processed water for up to 3 hours. The restoration of motility of the cell was estimated by observing the motile cell number using a video-microscopy system. A remarkable recovery of cell motility was observed with the incubation system using Fe, Mn and Zn encaging zeolite. However, they did not show any recovery effect when they were treated with a chelator, Chelex-100.

Introduction

Some of minerals are important to exhibit cellular functions such as xenobiotic metabolism, intracellular signal transduction, energy metabolism and cell movement. In our laboratory we have been studying the mechanism of physiologically functional water that is prepared by various functional materials by using a phytoplankton *Euglena gracilis* Z as a model organism, i.e., detoxication of TBT-intoxicated cells by some functional water species. Earlier, we reported that some water species which was treated by

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charcoal (wood ceramics) and Al-Fe ceramics, etc. promoted the excretion of tin compounds from tributyltin (TBT)-intoxicated *Euglena* cells, followed by enhancement of regeneration of flagellum and finally accelerated restoration of cell motility[1-4]. Previous results have suggested that minerals play critical roles in restoration of the intoxicated cells. In the last symposium we reported the effect of zeolite-treated water species on the restoration of the *Euglena* cells. Zeolite is micro porous aluminosilicate used as an industrial catalyst or adsorbent[4]. Parton *et al.*[5] reported that iron encaging zeolite-Y exhibited cytochrome P-450 like activity in combination with phthalocyanin. Their report made us to take interested in examining restoration of cell motility on the TBTCl-intoxicated *Euglena* cells whether water species treated with mineral-encaging zeolite could exhibit any favorable effect on detoxication of the cell *in vivo*.

In the present study, we report an invigoration effect of some mineral-encaged zeolite and their effect could be explained by the participation of specific minerals.

Materials and Methods

Examined mineral-encaged zeolite

As reported in the previous report[4], zeolite type-A and its mineral-encaged zeolite (Fe, Zn, Mn, Ca, Mg and Cu -encaged zeolite) were prepared by the Laboratory of Sinanen Zeomic Co., Nagoya. Five % (w/w) ratio of zeolite was suspended in distilled water for 15 min, then filtered through 0.45 μm Millipore® filter.

Organisms

A wild strain *Euglena gracilis* Z was cultured in Koren-Hutner medium[6] at 28℃ under illumination (2800 lux) with 12 hours light/dark intervals for 7 days was used for the TBTCl intoxication and subsequent restoration experiments.

Evaluation of restoration of cell morphology and motility

Restoration of cell morphology and motility was examined by video-microscope equipped with image analyzing system. *Euglena* cells were intoxicated by exposing to 50 μM TBTCl at final concentration for 1 min. After washing with the processed zeolite-treated water species for 3 times, *Euglena* cells in cyst form (spherical form) were suspended in the zeolite-treated water species in a plastic micro tube and incubated for 3 hours at 28℃ under illumination (2800 lux). At regular intervals of time, morphology and motility of the cells were examined microscopically under inverted microscope (Olympus, IMT-2) equipped with video image analyzer (ARGUS-100, Hamamatsu Photonics Co. Ltd., Hamamatsu, Shizuoka, Japan). By using Optimas data processing software, motile cell (spindle form) was counted on the video images, then the restoration efficiency was calculated by dividing motile cell numbers against total cell numbers in at least 10 different images. The restoration efficiency was compared with time. The data were treated by Q-test and student's t-test.

Analysis of elements in zeolite-treated water species

The composition of elements in the zeolite-treated water species was analyzed by inductivity-coupled plasma atomic emission spectrometry using Japan Jarrell-Ash model 96-953 according to the analytical condition of Sugiyama *et al.*[7)]

Results and Discussion

Recovery of cell motility by mineral-encaged zeolites

Figure 1 shows the recovery of cell motility of TBTCl-intoxicated *Euglena gracilis* Z by mineral-encaged zeolite treated water species. A remarkable recovery effect was observed for the *Euglena* cells incubated in the zeolite-treated water that was produced with zeolite-encaged Fe, Zn, Mn, Ca and Mg. The recovery effects by zeolite-encaged Fe, Zn, Mn, Ca and Mg were 57.2±8.9%, 55.8±8.2%, 48.1±7.5%, 20.2±7.3% and 19.3±6.9%, respectively. These values were significantly different.

Meanwhile, comparing with the control data, Cu-encaged zeolite treated water rather inhibited the recovery. Na-encaged zeolite, or zeolite type-A that was provided as the material for mineral encaged zeolite, did not show any recovery effect. The recovery effects by Cu and Na-encaged zeolite were

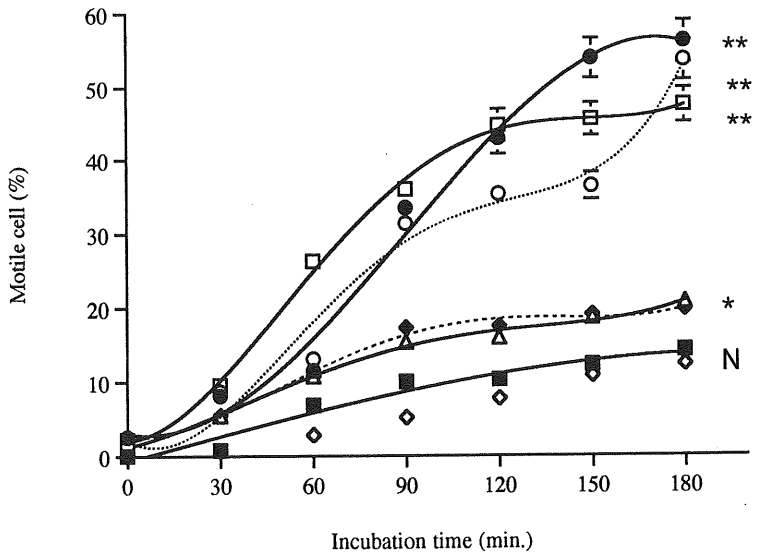

Fig. 1. Cell motility recovery of TBTCl-intoxicated *Euglena gracilis* in zeolite treated water (i.e., treated with zeolite-encaged Fe, Zn, Mn, Ca, Mg and Cu). Motility percent was calculated dividing motile cell numbers by the total cell numbers. Data presented are average values ±S.D. calculated from different 10 microscopic fields, or n=10. ** : $p<0.01$, * : $p<0.05$, ^{N} : no significance between the control data.
■ Incubated in the control ultrapure water; i.e., TBTCl-intoxicated cells were incubated in the ultrapure water ; ● zeolite-encaged Fe ; ○ zeolite-encaged Zn ; □ zeolite-encaged Mn ; △ zeolite-encaged Ca ; ◆ zeolite-encaged Mg ; ◇ zeolite-encaged Cu

11.3±4.3% and 13.8±2.3%, respectively. These values are not significantly different from the control, indicating no cell motility restoration effect.

Effect of chelator on the water species

We examined whether their restoration effect could be blocked by treating those zeolite-treated water with a chelator prior to incubation with TBTCl-intoxicated *E. gracilis* Z. As shown in Figure 2, treatment with one of the most effective chelator, Chelex-100 on the water species processed by the Fe, Zn and Mn-encaged zeolite, these water species lost their recovery effects (data with Zn and Mn-encaged zeolite are not shown in Fig. 2).

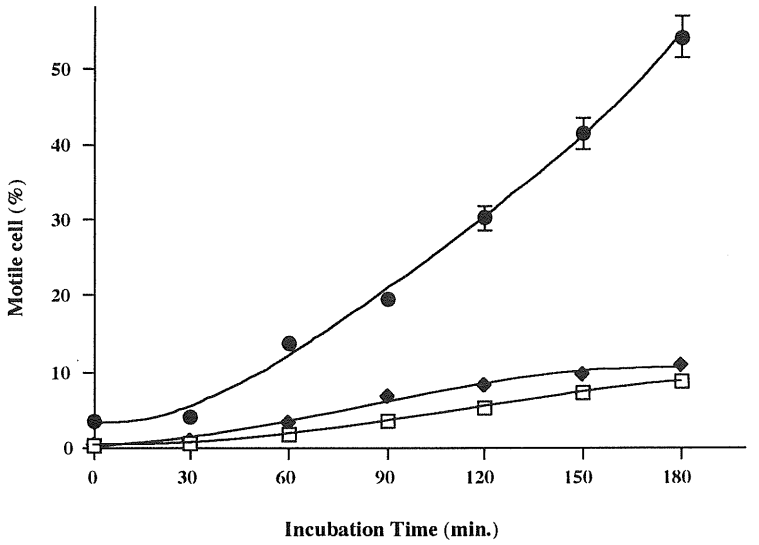

Fig. 2. Cell motility recovery of TBTCl-intoxicated *Euglena gracilis* in zeolite-encaged Fe and chelated zeolite treated water.
□ control ; ◆ chelated zeolite-treated water ; ● zeolite treated water

Analysis of elements in the processed mineral-encaged zeolite

As mentioned above, zeolite in the water seems to take part in the restoration of the cell motility; major elements in the water treated with Fe-encaged zeolite were analyzed by ICP. As shown in Figure 3, major elements in the water species treated with zeolite and major elements in the treatment with a chelator, Chelex-100, are presented. The principle components of water species treated with mineral-encaged zeolites were those found in encaged minerals (such as Fe, Zn, Mn, Ca, Mg and Cu), silica and aluminum.

Participation of minerals in the recovery of cell motility

It is known that iron is important for cellular roles in exhibiting the activity of the phase I biotransformation enzyme, cytochrome P-450, and calcium is also playing important roles as second messenger in intracellular signal transduction. To examine if the intoxicated *Euglena* cell motility would be restored by

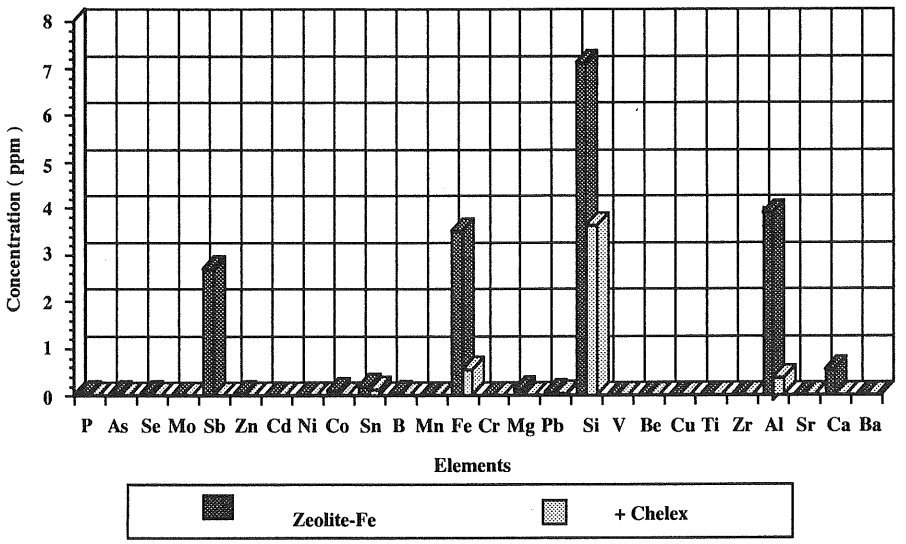

Fig. 3. Major elements in the water species treated with zeolite-encaged Fe and chelated zeolite treated water
Major elements in the zeolite treated water were measured by ICP.

the addition of the major minerals, equivalent concentration of Fe (as $FeCl_3$) and Ca (as $CaCl_2$) were added to the incubation system for the processed water species. Figures 4 and 5 show that their addition did not show any effect on the restoration of cell motility in the TBTCl-intoxicated *Euglena* cells.

Teo et al.[8] has reported on the mechanism of heavy metals such as organotin and their toxicity. It is also clear from our studies that some of trace minerals play important roles in restoring cell motility[2-4].

In the intoxication of *Euglena* cell by TBT, TBT is put into the organelle or cytosol, quickly. Then the stress of TBT causes the change of *Euglena* cell morphology as a protective reaction. And it is known that GTP-binding protein, phosphatidylinositol 4,5-bis phosphate and Ca^{2+} ions shared a signal transduction act on the biological response of stress.

During the detoxication process, exclusion of TBT out of the cell is necessary after transforming TBT more hydrophilic by cytochrome P-450. In the second step, the activation of signal transduction by Ca is needed to regenerate the flagellum in the cell. For the cell movement, Mg should participate to accelerate myofibrils, or contraction of pellicles.

Standing on the basis of data in the present study, these results suggest that the recovery effect can be led by Ca, Mg, Fe, however, it may not simply be attributable to minerals alone or adsorption of toxic metals by zeolite, but three dimentionally structured mineral and water clathlate formed by zeolite may also take part.

Although the detailed mechanism of the recovery effect of Fe, Zn and Mn-encaged zeolites-treated water species has not yet been elucidated, they should participate to help detoxication of the toxic TBTCl

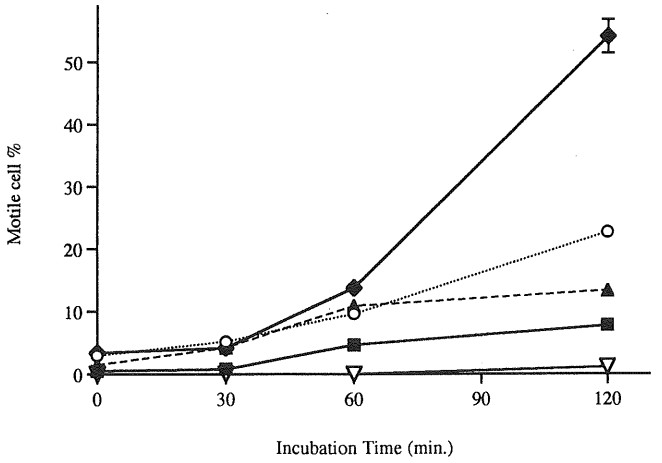

Fig. 4. Cell motility recovery of TBTCl-intoxicated *Euglena gracilis* in $FeCl_3$ and zeolite-encaged Fe
■ control ; ○ 0.1 ppm of $FeCl_3$; ▲ 1 ppm of $FeCl_3$; ▽ 10 ppm of $FeCl_3$; ◆ zeolite treated water

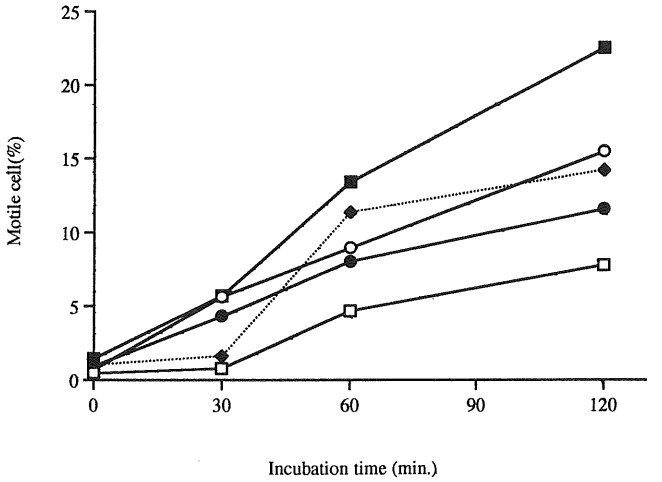

Fig. 5. Cell motility recovery of TBTCl-intoxicated *Euglena gracilis* in $CaCl_2$ and zeolite-encaged Ca
□ control ; ◆ 0.1 ppm of $CaCl_2$; ● 1 ppm of $CaCl_2$; ○ 10 ppm of $CaCl_2$; ■ zeolite treated water

in and/or on the surface of the cell.

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