Effect of oyster extracts on the recovery of cell motility of TBTCl-intoxicated *Euglena gracilis* Z

Tetsuya Suzuki[1], Mari Ohta, Kaoru Nakamura*, and Kozo Takama

(*Laboratory of Food Wholesomeness, Department of Marine Bioresources Chemistry, Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido 041, Japan; phone: + 81-138-40-5564; e-mail, ted @pop.fish.hokudai.ac.jp; * Institute of Chemical Research, Kyoto University, Uji, Kyoto, Japan*)

Abstract

Since oyster is an excellent source of minerals and taurine, it has been regarded as a health food. The authors have studied the biological role of trace minerals on the detoxification of xenobiotics by using tributyltin chloride (TBTCl)-intoxicated *Euglena gracilis* Z. In the present study, we examined detoxification effects by evaluating the recovery of cell motility for different kinds of oyster extracts; i.e., hot water extract of whole oyster flesh, extract rich in low molecular wt. fraction, extract rich in high molecular wt. fraction, and antioxidant rich fraction. After intoxication with 50 μM TBTCl, *E. gracilis* Z cells were washed with aqueous solutions containing 4 different oyster extracts separately at the dilution ratio of 100-100,000-fold, then incubated for 0-180 minutes at 28℃ under illumination (2800 lx). Recovery of cell motility was examined under the video microscope. Results showed that hot water extract at 1,000-fold dilution and the extract rich in the high molecular wt. fraction at 10,000 to 100,000-fold dilution was most effective on the recovery of cell motility. Since the treatment with a chelator, Chelex-100, suppressed the recovery of cell motility by trapping Ca, Mg and Fe in the solution, these minerals seem to have participated in the detoxification of TBTCl and/or the recovery of cell motility.

Introduction

Oyster is a well known edible bivalve mollusc belonging to the family, *Crassostrea* sp., and it has been eaten worldwide for a long time. Besides its good taste, oyster extract has been believed an excellent source of health promoting factors[1,2]. In fact, oyster extract is extremely rich in Zn[3,4], taurine and glycogen any of which are important nutrients for us. Oyster extract exhibits various physiological func-

1) To whom all the correspondence should be mailed.

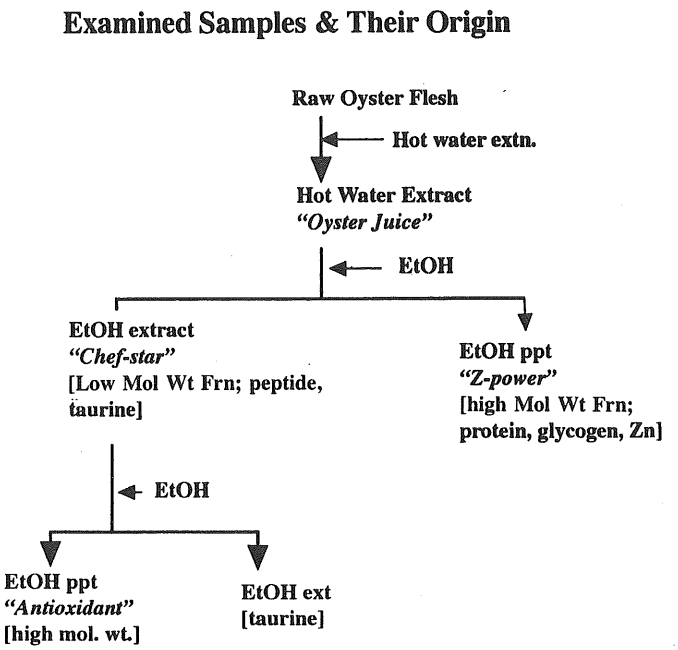
tions; e.g., activation of nerve tissue[5], suppression of platelet aggregation, tumoricidal effect[6], prevention and treatment of loss of taste sense. Since the oyster extract is rich in taurine and other sulfur containing substances[7], it exhibits antioxidant activity or detoxification activity. Recently, Tapiero and Tew reported increased glutathione expression in cells induced by *Crassostera gigas* extract (JCOE)[8]. Thus oyster extract has been regarded as a health food material that can prevent degenerative diseases. Furthermore, it reportedly helps to activate hepatic function. However, definite evidence has not been shown yet.

In the present study, we attempted to evaluate the detoxification effect of oyster extract by detoxification evaluation assay system using TBTCl-intoxicated Euglena cells and their cell motility recovery.

Materials and Methods

Examined oyster extracts

We examined 4 different oyster extracts all of which were kindly supplied by Japan Clinic Corp., Kyoto, Japan. In Scheme 1 the preparation procedure is outlined. According to the information from the Central Laboratory of Japan Clinic Corp., they are hot water extract (trade name, "Oyster Juice"), low molecular weight fraction ($<10^4$ dalton) which is rich in peptide and taurine (trade name, "Chef-star"),



Scheme 1. Procedure for the preparation of oyster extracts
Because of business secret for Japan Clinic Corp., detail procedure for the preparation of each oyster extract is not opened to the public.

high molecular weight fraction ($>10^4$ dalton) which is rich in protein, glycogen, and Zn (probably bound to organic substance) (trade name, "Z-powder"), and fraction rich in antioxidant (trade name, "Antioxidant"). Because of know-how in the manufacturing process, detail information on each extract has not been available.

In the present paper, we used abbreviation "Hot Water Extract" for the fraction with the trade name "Oyster Juice", "Low Molecular Extract" for the low molecular fraction with the trade name "Chef-star", "High Molecular Extract" for the high molecular fraction with trade name "Z-powder", and the trade name "Antioxidant" for "Antioxidant".

Those samples were provided as liquid concentrate except for "High Molecular Extract". The "High Molecular Extract" was provided as freeze-dried powder. All of the extracts except for the "High Molecular Extract" were dissolved in the ultrapure water at the concentration of 250 and 1,000-fold volume of dilution. The "High Molecular Extract" was suspended in the ultrapure water at the concentration of 10ppm and 100ppm.

In order to examine the participation of minerals the water species prepared by diluting different oyster extracts were used in the assay system using *Euglena gracilis* Z. As the control water species, ultrapure water was prepared by doubly distilled, then filtered through Chelex-100 column to remove minerals. In case of Chelex-100 treatment, oyster extract solution was run on the column filled with Chelex-100 to remove divalent minerals such as Fe, Ca, Mg, and Zn.

Euglena cell strain

Euglena gracilis Z grown on the Koren-Hutner medium[9] at 28℃ under illumination (2800 lx) with 12 hours light-dark intervals for 7 days was used for the TBTCl intoxication and subsequent recovery experiments.

Evaluation of restoration of cell morphology and motility on TBTCl-intoxicated Euglena cells

After incubating *Euglena gracilis* Z for 7 days, cells were harvested and provided for TBTCl intoxication as described in our previous paper[10].

Recoveries of cell morphology and motility were examined by video microscopy as reported earlier.[4,5] Namely, Euglena cells were intoxicated by contacting to 50 μM TBTCl at final concentration for 1 min. After washing with the solution containing oyster extract for 3 times, Euglena cells in cyst (spherical) form were suspended in the water species containing oyster extract at the dilution rate of 250 and 1,000-fold or 10 and 100 ppm for the "High Molecular Extract" in an Eppendorf tube, and incubated for 180 min at 28℃ under illumination (2800 lx). At regular time interval, cells were examined microscopically under inverted microscope (Olympus IMT-2) equipped with video image analyzer (ARGUS-100, Hamamatsu Photonics, Hamamatsu, Japan). The number of motile cell (or spindle) form was counted on the video images, then recovery efficiency as % was calculated dividing motile cell numbers by total cell numbers in at least different 10 image frames. The recovery efficiency was compared

with time.

Analysis of elements in processed water species

Composition of elements in the diluted oyster extract solutions was analyzed by inductively-coupled plasma atomic emission spectrometry using Japan Jarrell-Ash model 96-953 according to the analytical condition of Sugiyama et al.[11)]

Results and Discussion

Recovery of cell motility by oyster extracts

The effect of 4 different kinds of oyster extract on the recovery of cell motility of TBTCl-intoxicated Euglena cells are presented in Figs. 1-4. More or less in each case some extent of recovery of cell motility was recognized at 180 min of incubation, however, there were considerable differences in the recovery efficiency. Except for the "High Molecular Fraction", any significant difference between the examined sample and the control was not recognized up to 150 min of the incubation. On the effect of the "Hot Water Extract", significant recovery was recognized at the dilution of 1000 times (Fig. 1). The "Hot Water

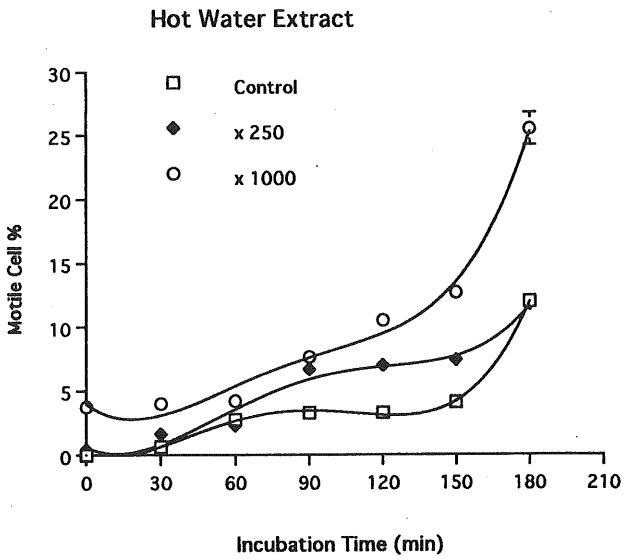


Fig. 1. Effect of "Hot Water Extract" at the concentration of 250-fold volumes of dilution and 1,000-fold volumes of dilution on the recovery of TBTCl-intoxicated Euglena cells

X-axis represents incubation time after treatment with the extract, and Y-axis cell motility represented as % of the total cell number.

◆ x 250 Euglena cells incubated in the "Hot Water Extract" fraction incubated at 250-fold volumes of dilution.

○ x 1,000 "Hot Water Extract" fraction incubated at 1,000-fold volumes of dilution.

□ Control: Incubated in the ultrapure water.

Plots represent (mean ± S.D.) for n=10. (Same for Figs. 2-4)

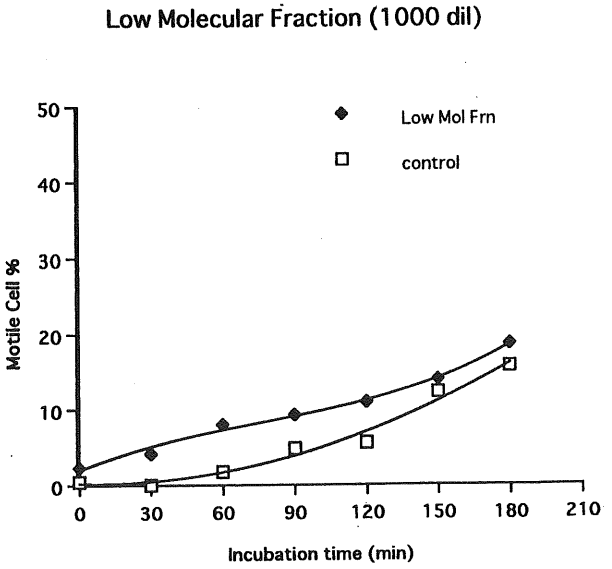


Fig. 2. Effect of low molecular weight fraction on the recovery of cell motility of TBTCl-intoxicated Euglena cells

◆ Low Mol Frn: Euglena cells incubated in the "Low Molecular" weight fraction at 1,000-fold volume of dilution.

□ Control: Euglena cells incubated in the ultrapure water

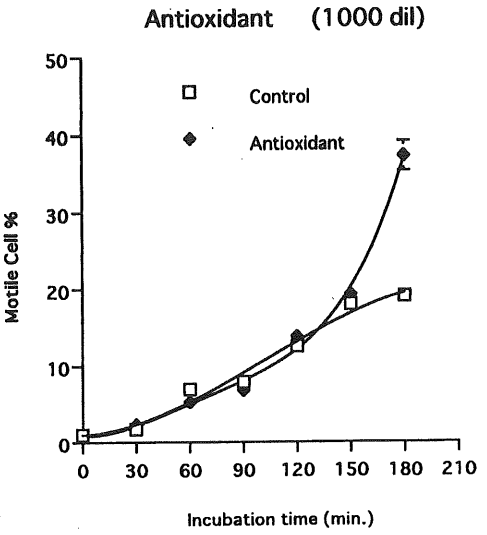


Fig. 3. Effect of antioxidant fraction on the recovery of cell motility of TBTCl-intoxicated Euglena cells

◆ Antioxidant: Euglena cells incubated in the "Antioxidant" fraction at the concentration of 1,000-fold volume of dilution.

□ Control: Euglena cells incubated in the ultrapure water.

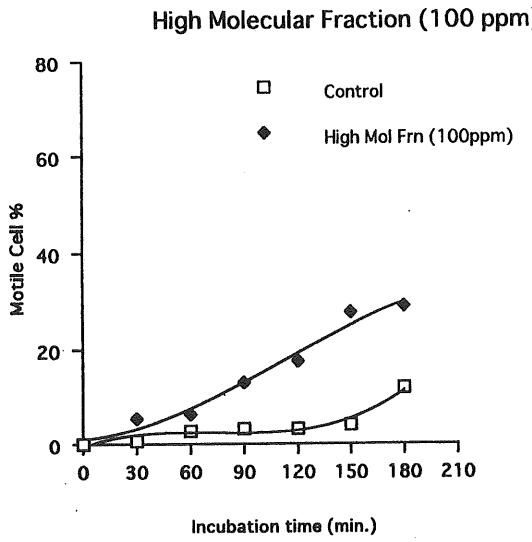


Fig. 4a. Effect of high molecular weight fraction on the recovery of cell motility of TBTCl-intoxicated Euglena cells

◆ High Mol. Frn (1000 dil): Euglena cells incubated in the "High Molecular Weight Fraction" at the concentration of 100 ppm.

□ Control: Euglena cells incubated in the ultrapure water

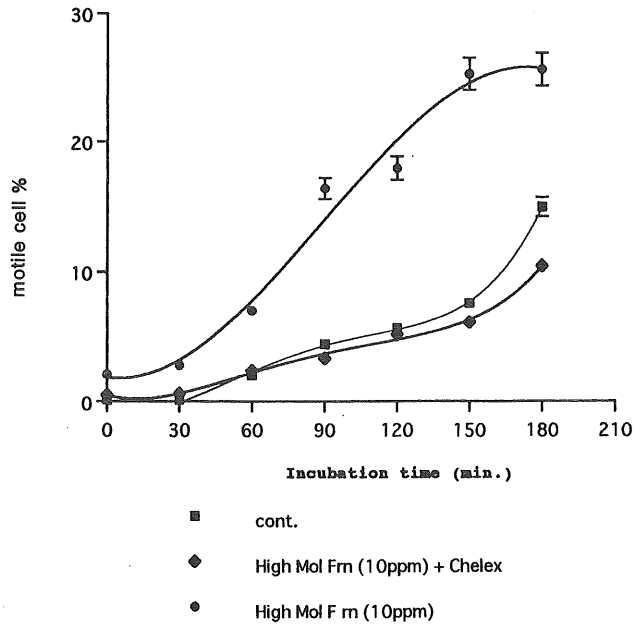


Fig. 4b. Comparison of cell motility recovery effect of "High Molecular Weight Fraction" and Chelex-100 treated "High Molecular Weight Fraction"

● : Euglena cells incubated in the solution of "High Molecular Weight Fraction" at the concentration of 10 ppm

◆ : Euglena cells incubated in the solution of the Chelex-100 Treated "High Molecular Weight Fraction"

■ : Euglena cells incubated in the ultrapure water (control)

Extract" at the dilution of 250 times, on the other hand, did not show any significant recovery effect.

Low molecular weight fraction which is composed of peptide and taurine did not show any significant difference from the control in cell motility recovery at 1000 times dilution.

"Antioxidant" fraction showed significant recovery after 180 min incubation at the dilution of 1000 times.

"High Molecular Fraction" was significantly effective at the concentration of both 10ppm and 100ppm (Fig. 4a, b).

Participation of minerals in the recovery of cell motility

In the case of motility recovery of TBTCl-intoxicated Euglena cells by known functional materials, their functions were mostly due to trace minerals especially Ca and Mg[9,12,13]. If the recovery effect of the oyster extract would also be due to trace minerals as recognized in our earlier study using "Functional Materials"[11-14], its effect should be suppressed by the treatment with chelator. Treating the solution of "High Molecular Fraction" with a strong chelator, Chelex-100, totally suppressed its effect (Fig. 4b). Although some recovery was observed in the control and Chelex-100 treated solution after 180 min of incubation, their recoveries were far less than that of the "High Molecular Fraction" solution at 180 min.

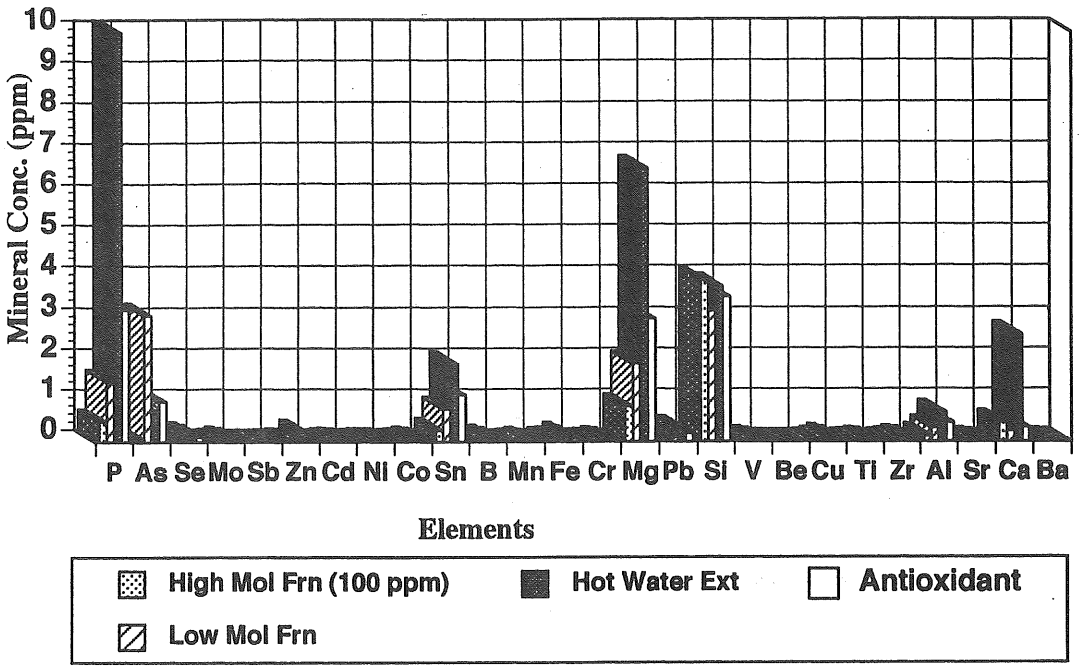


Fig. 5. Contents of elements in the oyster extract solution determined by ICP-AES.
Low Mol Frn: "Low Molecular Weight Fraction" at 1,000-fold volume of dilution, High Mol Frn: "High Molecular Weight Fraction" at 100 ppm, Hot Water Ext: "Hot Water Extract" Fraction at 250-fold volume of dilution, Antioxidant: "Antioxidant" at 1,000-fold volume of dilution

The result indicates that the recovery effect should be due to mineral(s).

Comparing the composition of elements in the oyster extracts, P, Mg, Si, and Ca were detected as the major components (Fig. 5). Besides them, As, Sn, and Al were present. P, Mg, Si, and Ca were detected at ppm level in the "Hot Water Extract" fraction. P, Mg, and Ca contents in the "Hot Water Extract" fraction were much higher than other extracts, while As was high in the "Low Molecular Fraction". Si was detected as a common major element. Strangely, Zn, that should be detected as extraordinary high content in every sample or at least in the "High Molecular Fraction", was present as a minor constituent at 26 ppb. Zn contents in other fractions were also at ppb level ranging 20-240ppb. The highest content of Zn was detected in the "Hot Water Extract". According to the food composition table, Zn content in the oyster is at the range of 400ppm in the edible part of raw oyster. Similarly, the contents of Fe (0.18ppm) and Mg (6.68ppm) in the "Hot Water Extract" were far lower than the data presented in the food composition table; i.e., Fe (36ppm) and Mg (700ppm). The reason for extremely low Zn and other mineral concentrations has not been clearly explained yet, however, it is probably due to the reason that those solutions were directly subjected to ICP analyses without heat digestion with strong acid. The direct injection without acid digestion sometimes enhances the background in the ICP analysis. Since Zn

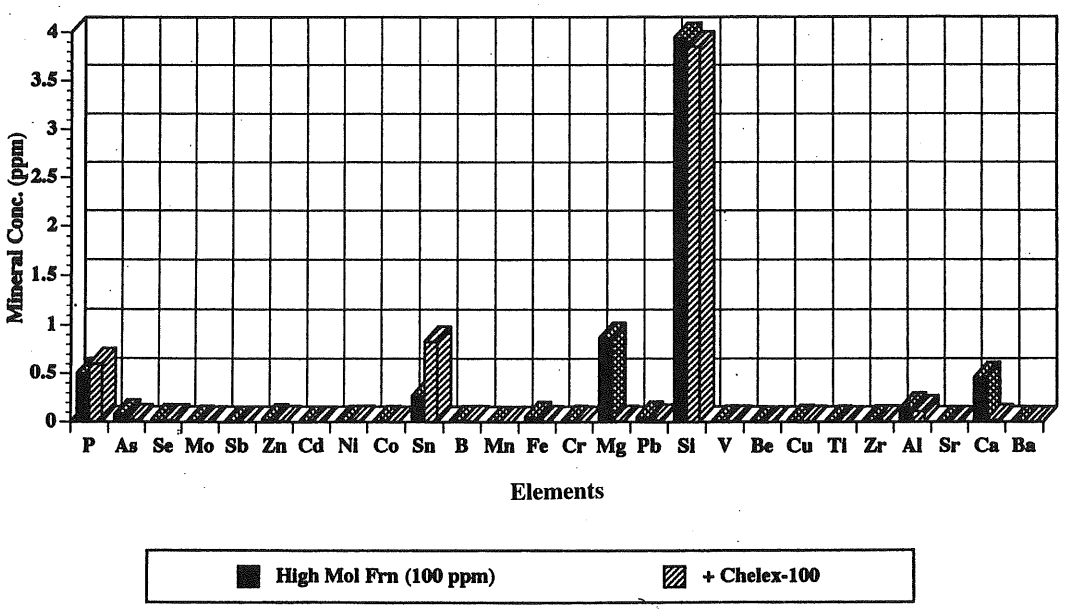


Fig. 6. Comparison of changes in the contents of the major elements in the "High Molecular Weight Fraction" and Chelex-100 treated "High Molecular Weight Fraction" determined by ICP-AES.

High Mol Frn: Contents of major elements in the "High Molecular Weight Fraction" at the concentration of 100 ppm, + Chelex-100: Contents of major elements in the Chelex-100 treated "High Molecular Weight Fraction"

in oyster flesh is thought to be present in organometallic form combining to high molecular compounds[15], it is likely that Zn content in organometallic form may have made it difficult to carry out correct analysis of Zn by increasing background noise in ICP analysis. Anyway, cross check analysis by atomic absorption after acid digestion of the oyster extracts should be made.

Inhibitory action of the "Hot Water Extract" at 250-fold dilution may be explained by excess of As (0.73ppm), Sn (1.92ppm), Mg (6.68ppm), Cu (0.13ppm) , and Ca (2.65ppm). Adverse effect of the "Low Molecular Fraction" may be due to excess of As (3.11ppm) and Sn (0.8ppm).

Treatment of the "High Molecular Fraction" with the chelator, Chelex-100, definitely reduced contents of Mg (0.87ppm to 0.01ppm), Ca (0.46ppm to 0.02ppm), Fe (0.05ppm to 0.01ppm), and Zn (0.026ppm to 0.01ppm) (Fig. 6).

No change in the contents of Si, Se, Al, and Cu was observed before and after Chelex-100 treatment. Increase in Sn (0.27-0.83ppm) may be due to artifact. From these results, detoxification of TBTCl and recovery of cell morphology and motility of TBTCl-intoxicated Euglena cells by the oyster extract can also be explained by any of Mg, Ca, Fe, and Zn. How and to what extent do the elements in the extracts mentioned above participate in the recovery has remained unanswered. Detailed study should be run on for the further study.

Acknowledgments

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