Comparative study on the growth inhibition and metabolism of sodium arsenite in *Euglena gracilis* strains Z and SMZ

Akiko Suzuki[1], Toshikazu Kaise[2], Masumi Watanabe[1] and Tetsuya Suzuki[1]

[1] *Hokkaido University, Graduate School of Fisheries Sciences, Division of Life Sciences*;*

[2] *Tokyo University of Pharmacy and Life Sciences***

Summary

Animals and plants differently respond to inorganic arsenite. In the present study, the authors examined the toxicity of sodium arsenite on the growth of an algae *Euglena gracilis* Z and its achlorophyllous mutant SMZ strains. The metabolism of sodium arsenite in Z and SMZ strains was also investigated by HPLC/ICP-MS. Comparing cell growth, Z strain was retarded under 0.25 to 0.5 mM, while the growth of SMZ retarded above 1mM indicating difference of cellular response between photosynthetic strain and non-photosynthetic strain. HPLC/ICP-MS analysis identified methyl arsenic acid, dimethyl arsenic acid, and trimethylarsine oxide in both the strains suggesting similar metabolic pathway in *Euglena gracilis* as reported in higher organisms.

Introduction

Arsenic is present as inorganic forms such as FeAsS, AsO_4^{3-} in earth crust and underground water[1]. While arsenic content in marine organisms especially in seaweeds is rich, and they are present as arsenobetaine, arseno sugar and arsenocholine[2]. The toxicity of arsenic remarkably differs by its chemical forms; i.e., inorganic arsenic is usually highly toxic[2], but organic form one is not so toxic or no toxicity. Arsenic is known as a trace nutrient participating in methionine metabolism[3]. Studies on the metabolism of arsenic by unicellular organisms in aqua-sphere have been done with *Chlorella*[4]. Inorganic arsenic compounds are metabolized to form organic forms as shown in Fig. 1.

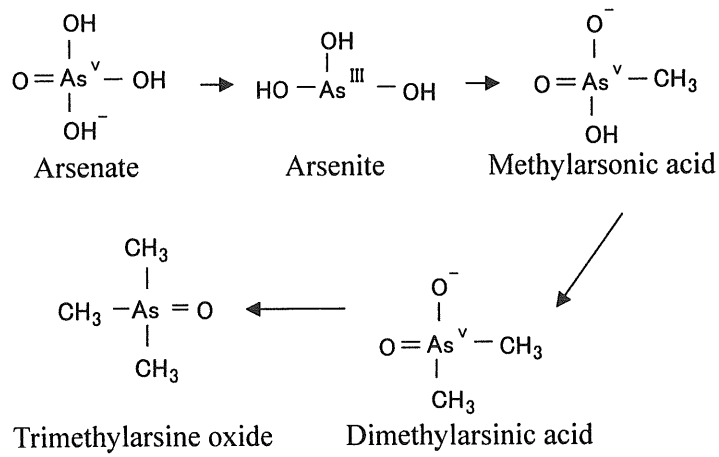

Fig. 1 Metabolic pathway of inorganic arsenic in higher organisms.

* Address : Corresponding author : 3 - 1 - 1 Minato, Hakodate 041 - 8611, Hokkaido, Japan

** Address : 1432 - 1 Horinouchi, Hachioji, Tokyo 192 - 0392, Japan

We have used a unicellular eukaryote *Euglena gracilis* Z and its achlorophyllous mutant SMZ for toxicological studies of environmental chemicals[5]. However, we have not examined the toxicity and metabolism of inorganic arsene on *E. gracilis* Z and SMZ yet. The present study was undertaken to examine the effect of sodium arsenite and its metabolism on *E. gracilis* Z and SMZ. We obtained data indicating sodium arsenite suppressed cell growth but not fatal, and was transformed to organic arsene as reported in other algae and higher organisms (Fig. 1).

Materials and Methods

Preparation of the inorganic arsenic

Sodium arsenite ($NaAsO_2$) purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) was dissolved in Koren Hutner (KH) medium to prepare different concentrations of arsenic[6]. All other chemicals used were guaranteed reagent grade.

Cell cultivation and arsenic exposure

A wild strain *Euglena gracilis* Z and its achlorophyllous mutant SMZ were cultivated in a KH medium at 28℃ under illumination (2800 lx) with a 12-hour light-on-off interval for 7 days. Cells at early stationary phase were used for the following experiments.

Effect of $NaAsO_2$ on the cell proliferation

For assessing the effect of sodium arsenite on the growth of *Euglena gracilis* Z and SMZ cells were incubated with sodium arsenite of different concentration ranging from 0.00175mM to 1.75mM.

The cell growth was monitored by measuring the turbidity at 610nm with time on a Bosch and Lomb spectrometer (Shimadzu Instruments, Kyoto, Japan) over 10 days.

Sample preparation for HPLC / ICP-MS

Cultivated cells were washed with distilled water. Then, the harvested cells were disrupted by ultrasonication. Disrupted cell suspension was centrifuged at 14,000 × g for 20min at 6℃ to precipitate cell debris. Supernatants were filtered through 0.22 μm membrane filter to obtain filtrates. Filtrates thus obtained were provided for the analysis of metabolites of sodium arsenite by HPLC / ICP-MS.

The measurement of the intracellular metabolite

The metabolites of sodium arsenite were identified by the arsenic analysis system according to Kaise et al. using HPLC / ICP-MS[7].

Results and Discussion

Effect of sodium arsenite on the cell growth

Growth of *E. gracilis* Z in the Koren-Hutner liquid medium containing higher than 0.25 mM sodium arsenite was suppressed, however, no growth suppression was observed below 0.25 mM (Fig. 2). On the other hand, the growth of *E. gracilis* SMZ was suppressed by sodium arsenite above 1 mM (Fig. 2). Those data suggest that cellular response to sodium arsenite is different between Z and SMZ strains.

Comparing viable cells between Z and SMZ strains under arsenic stress loading, population of dead cell did not increase by sodium arsenite exposure so far as examined in the present study (data not shown). In other words,

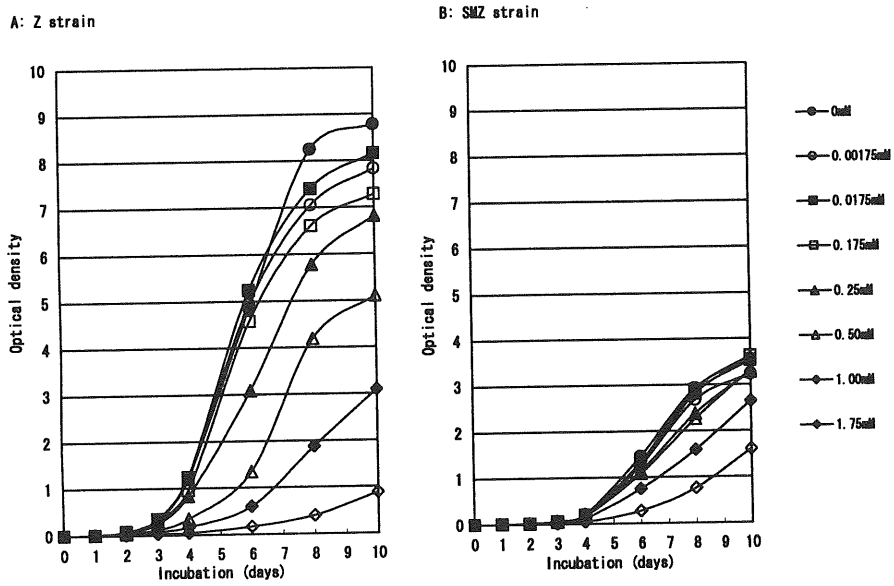

Fig. 2 Effect of sodium arsenite exposure on the cell growth of *Euglena gracilis*. Control, growth of E. gracilis without sodium arsenite exposure under 12/12h light dark cycle at 28℃ for 10 days. Cell growth was monitored at 610 nm.
Arsenic concentration (mM): ●, 0; ○, 0.00175; ■, 0.0175; □, 0.175; ▲, 0.25; △, 0.50; ◆, 1.00; ◇, 1.75.

growth suppression by sodium arsenite was not due to cell death but retardation of cell proliferation.

By the HPLC/ICP-MS any arsenic compounds were not detected in the filtrate prepared from ***E. gracilis*** cells without sodium arsenite exposure. Arsenite, methylarsonic acid and dimethylarsinic acid were detected in the Z strain incubated for 48 h and 72 h in the KH medium containing 0.20 mM sodium arsenite. Arsenite and dimethylarsinic acid were detected from Z strain and SMZ strain incubated for KH medium containing 0.20 mM sodium arsenite for 18 h (Fig. 3).

These data suggest that ***E. gracilis*** strains Z and SMZ have metabolic pathway similar to that of higher organisms. Arseno sugar was identified in ***E. gracilis*** Z (data not shown). Detailed study on the identification of organic arseno compounds is now under way.

References

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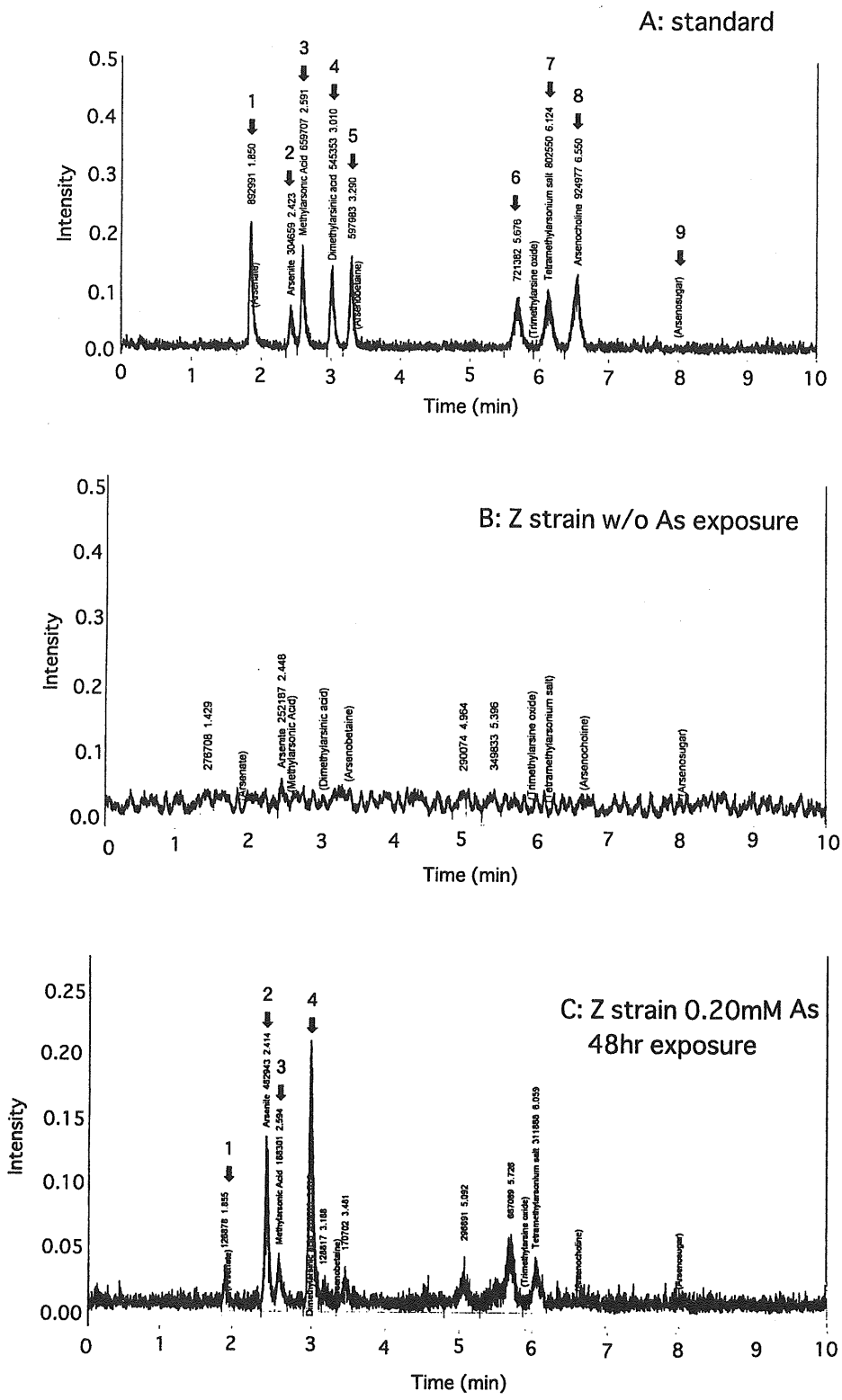

Fig. 3 The total ion chromatograms of arsines in *Euglena gracilis*. (A) HPLC/ICP-MS chromatogram of arsenic standard compounds. (B) Chromatogram of *Euglena gracilis* Z without sodium arsenite exposure. (C) Chromatogram of *Euglena gracilis* Z with 0.20mM sodium arsenite exposure for 48 hours. Numerals in the chromatograms represent: 1, Arsenate ; 2, Arsenite ; 3, Methylarsonic acid ; 4, Dimethylarsinic acid ; 5, Arsenobetain ; 6, Trimethylarsine oxide; 7, Tetramethylarsonium salt ; 8, Arsenocholine.