

## Effect of iron and aluminum on growth of unicellular eukaryote *Euglena gracilis* Z

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### Summary

Iron (Fe), an important essential trace element, causes oxidative stress in excess. Aluminum (Al), one of the most abundant elements in nature, also impairs plant growth. Al is recently suspected to cause degenerative nerve diseases in mammals. Al has been reported to enhance Fe-induced oxidative injuries in mammals. The present study was undertaken to assess whether similar adverse effect would be observed in the unicellular algae and protist, *Euglena gracilis* Z. The effect of single or combined exposure of Fe and Al from 0 to 5.0 mM on cell growth was examined. In comparison to the growth of *E. gracilis* in the Koren-Hutner medium [KH] containing 0.16 mM Fe<sup>2+</sup> as the control, that in the KH without Fe was ca 25% less than the control. Addition of Fe<sup>2+</sup> from 0.31 to 5.0 mM inhibited the cell growth dose dependently. Meanwhile, single addition of Al<sup>3+</sup> from 0 to 5.0 mM also suppressed the growth above 0.16 mM, however, it was not dose dependent. Moreover, complex addition of Al<sup>3+</sup> and Fe<sup>2+</sup> from 0.16 to 5.0 mM for each element increased suppression of the cell growth than Fe and alone. Possible reasons for growth suppression by Fe and Al are discussed.

### Introduction

Iron (Fe) is an essential trace element for most organisms by playing roles in respiration system, biotransformation of xenobiotics, etc. Generally, Fe in biological systems exists as bound to proteins or heme complex. The Fe-binding proteins have many functions; e.g., oxygen carrier, oxygen storage and electron transfer<sup>1)</sup>. On the other hand, Fe ion liberated from decomposed protein is not precipitated at physiological pH because it forms chelated complex in the biological system. Therefore, in some cases Fe<sup>2+</sup> ion plays to generate ROS by reacting with molecular oxygen at physiological pH<sup>2)</sup>.

On the other hand, Al is one of the most widely distributing elements in nature especially in the soil. However, essentiality of Al for both the plants and animals has not yet been fully studied. The toxicity of Al to plant has been reported in acid soil area occupying nearly one-half of all nonirrigated, arable lands in the world<sup>3)</sup>. In mammals the essentiality of Al has not been proved yet, but rather its toxicity by accumulating in brain, bone<sup>4)</sup>, has been reported. Hirsch et al.<sup>5)</sup> and Good et al.<sup>6)</sup> reported that more Al and Fe were accumulated in the brain of patients suffering from neurological disorders such as Parkinson's disease and Alzheimer's disease. Other studies reported that Al accelerated formation of peroxides induced by Fe in ox-brain liposome<sup>7)</sup>, mice brain homogenate<sup>7, 8)</sup> and rat cultured neuron<sup>9)</sup>.

It is useful and important to examine whether similar excess inhibition would be observed in the combined exposure of aluminum and iron in plant kingdom especially in phytoplankton that supports food production and oxygen

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generation on the earth. We have used a characteristic unicellular organism classified as algae and protozoa, *Euglena gracilis* Z and its achlorophyllous mutant strain *E. gracilis* SMZ as tool organisms for toxicological studies especially of oxidative stress-induced cellular injury and its remedy by trace nutrients. We have presented many reports on cytotoxicity of organotin<sup>10</sup>, cadmium<sup>11</sup>, herbicide and ultraviolet<sup>12</sup> in connection with oxidative cellular injuries. In the present study, the effect of single and combined exposure of Al<sup>3+</sup> and Fe<sup>2+</sup> was examined on the growth of *E. gracilis* Z as plant model; i.e., cell growth under the different concentrations of Fe and Al was compared to assess their inhibitory effect. We observed excess inhibition of Fe<sup>2+</sup> and (Fe<sup>2+</sup>+Al<sup>3+</sup>) on the cell growth. Possible explanation on the growth suppression by Fe and Al is also discussed.

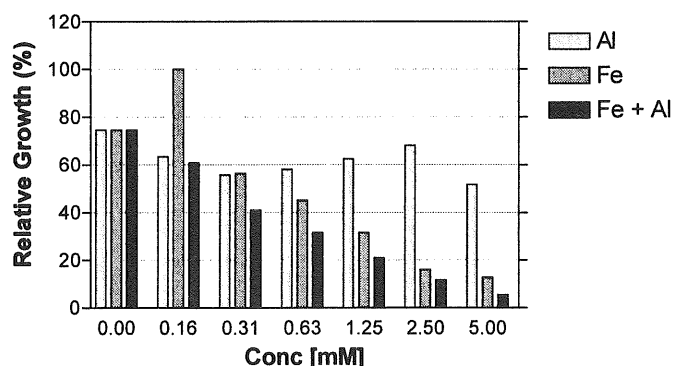
## Materials and Methods

### Model organism and culture medium

All the experimental procedure for cell culture was carried out aseptically. A wild type strain operating photosynthesis, *Euglena gracilis* Z, was cultured beforehand in Koren-Hutner (KH) liquid medium (pH 3.5)<sup>13</sup> for 7 days under illumination of 12 hours light/12 hours dark cycle (fluorescent lamp for plant growth: 79.5  $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) at 28 °C without aeration. The KH liquid medium contains 0.13 mM Fe that is required for cell growth as an essential trace nutrient; we set up the KH liquid medium as the control. Besides the control KH medium, media containing different concentrations of Fe by dissolving FeSO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O were prepared. Al<sup>3+</sup> medium and complex (Fe<sup>2+</sup>+Al<sup>3+</sup>) medium which contained same concentrations of Fe<sup>2+</sup> and Al<sup>3+</sup> were prepared by dissolving FeSO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O and AlNH<sub>4</sub>(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, respectively. One hundred ninety  $\mu\text{l}$  each of these media was dispensed into an each hole of 96 holes microtitration plastic dish. Ten  $\mu\text{l}$  each of *E. gracilis* Z cell suspension was inoculated into each hole, and then cultured for 5 days under the same condition as described for pre-culture. The cell growth was monitored by the change of the turbidity of cell suspension at 610 nm with Microplate Reader (TECAN SPECTRAFLUOR X/FLUOR, TECAN Austria GmbH).

## Results and Discussion

Figure 1 shows the growth rates of *E. gracilis* Z cell cultured under different concentrations of Fe ion and Al ion



**Fig. 1** Relative cell growth of *Euglena gracilis* Z in Koren-Hutner medium with different concentrations of iron (Fe) and aluminum (Al). Cell growth was compared after 5 days' incubation in Koren-Hutner liquid medium under different concentrations of iron and aluminum. Y-axis represents relative cell growth to the control KH medium; x-axis represents concentration of iron and aluminum. Data are mean of 5 measurements for each experiment. Experimental details are in the text.

in KH medium. The growth rate of control medium that contained 0.16 mM Fe ion but no Al ion was represented as 100%. Since *E. gracilis* Z cell requires Fe as an essential trace nutrient for the growth, the growth under the Fe deficient condition was ca 20% lower than the control KH medium. Cell growth was suppressed above 0.31 mM Fe showing inhibition by Fe excess.

Meanwhile 0.16 mM Al suppressed the growth by 30% of the control. However, suppression was not dose dependent up to 5.0 mM. The cell growth under the combined addition of (Al + Fe) was more inhibited than single Fe exposure.

Iron in *E. gracilis* Z is required for mitochondrial functions, photosynthesis, xenobiotics metabolism by cytochrome P450, and other redox reactions in tricarboxylic acid cycle. However, excessive addition of Fe definitely suppressed the cell growth dose dependently. The cause of iron-induced growth inhibition should be due to its transitional electron-transferring characteristic to produce ROS under aerobic condition, which damages intracellular components and impairs cellular functions. It is easily considered that Fe in the KH media kept at pH 3.5 was absorbed by the cells, however, we have not obtained direct proof of ROS involvement by Fe and Al in *E. gracilis* Z cells yet.

Considering the fact that Al is soluble under acidic condition to result in phytotoxicity, it is highly likely Al was solubilized and absorbed by the *Euglena* cell in the KH media kept at pH 3.5 in all cases. However, the physiological mechanism that Al damages plant cells including *E. gracilis* Z is not known yet.<sup>14)</sup>

The mechanism why the complex exposure showed more growth suppression also remains unexplained. Since the target subcellular organella of Al in *E. gracilis* Z is not known, we cannot show any concrete mechanism on the enhanced growth suppression.

To explain the increased growth suppression by combined exposure of Fe and Al, further studies with *E. gracilis* Z and medium should be made. Preliminary experiments suggest some possible reasons including involvement of oxygen-derived stress. One possible reason for the growth depression by single Fe addition and complex Fe and Al one may be due to lowering dissolved oxygen in the medium. Measuring dissolved oxygen concentration (DO) in the media showed that addition of Fe<sup>2+</sup> to the KH decreased DO in the medium dose-dependently, but addition of Al did not. The addition of Al to Fe<sup>2+</sup> did not change the DO as observed in single Fe<sup>2+</sup> addition indicating Al has neither additive effect in consumption of DO nor preserving DO level (data not shown). If the growth inhibition is due to short of oxygen supply, growth suppression will not occur under aeration. However, we should keep in mind that *E. gracilis* Z operates photosynthesis in the light to produce oxygen, and the cells consume oxygen for respiration. DO level measured in the preliminary experiment was merely obtained from model system without cell. Therefore, we cannot give any comment except that DO level was reduced by Fe<sup>2+</sup> addition to the medium. To verify the decrease of DO in the medium, further experiment should be made using precisely counted live cell population to express DO as [ $\mu$ M]/live cell. Precise measurement of DO in the presence of *E. gracilis* Z cells in the light and dark is now under way.

Second possible elucidation on the inhibition of cell growth may be because of ROS generated in the medium that altered or decomposed cyanocobalamin, thiamine and amino acids to lead nutrient deficiency. Analysis of medium composition especially organic nutrients should be needed. The third probable reason is that ROS generated in the vicinity of cell membrane altered membrane structure and functions. The fourth possible reason is intracellular ROS catalyzed by Fe and Al impaired cell function to grow. In aqueous solution containing FeCl<sub>3</sub>, we confirmed HO $\cdot$  generation by ESR-spin trapping technique. However, the data cannot be direct evidence of decomposing nutritional constituents of the medium by HO $\cdot$  nor intracellular HO $\cdot$  generation because HO $\cdot$  was only detected when FeCl<sub>3</sub>

was dissolved in ultrapure water (data not shown).

In the present study, we observed Fe and Al-induced growth inhibition of *E. gracilis* Z. Growth inhibition by Al was also observed but it was not so severe as Fe. Complex exposure of Fe and Al slightly promoted the inhibition of cell growth than separate single dose of Fe and Al. The mechanism of the growth inhibition is not known yet, however, the present study indicates small biota such as phytoprotozoa would be affected by complex Fe and Al exposure.

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