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Cadmium Toxicity in the Macrophytic Alga *Chara*: Role of Reactive Oxygen Species and Effect of Zinc

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INTRODUCTION

Cadmium (Cd) is a toxic heavy metal that contaminates many environments worldwide. We are developing the large, freshwater alga *Chara australis* as a possible biological agent for removing Cd from aqueous environments. Earlier work in our lab showed that *Chara* survives in sediment and waters containing cadmium and translocates to the harvestable shoot. We also showed that zinc (Zn) protects against Cd toxicity. This was indicated by survivorship. (Fig 1, 2)

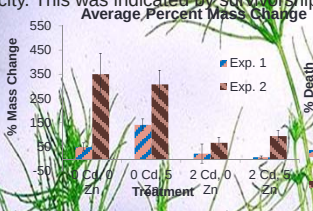

Fig 1: The average percent mass change for the 2 experiments are shown

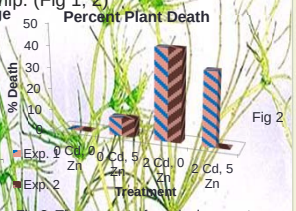

Fig 2: The percent of survival over a two week period

Fig 2

In the work reported here, we explore the nature of Cd toxicity and how Zn protects against it. We hypothesized that reactive oxygen species, ROS, which can cause oxidation damage to cellular components, were produced as a response to heavy metal stress. We developed a technique using a compound (DCHF-DA) that becomes fluorescent when oxidized to measure ROS levels. We confirmed that this technique was valid by exposing the algae to high light or wounding, known to generate ROS in other species, and showing increases in fluorescence. However, after 4 weeks incubation in Cd, fluorescence decreased. We hypothesize that the initial production of ROS in response to Cd stress is counteracted by the synthesis of anti-oxidant compounds, and that Zn protects against toxicity by enhancing the production of the compounds.

METHODS

24 hours before the experiment, plants were cut and placed in growth solution in a dark environment to allow recovery from the stress of cutting. On the day of the experiment, the plants' masses were recorded. Six plants per tube were incubated in 14.1nM of DCHF-DA in growth medium. Incubated plants were placed in a small fingerbowl containing a solution of 1mM CaCl2 +1mM MES solution. The bowl and its contents were shaken for 3-5 minutes. Plants were ground in liquid nitrogen and suspended in 1.25mL Tris-HCl buffer (40mM, pH 7.0), shaken for 5 min, and centrifuged for 10 min. Supernatant was diluted 1:2 in buffer and fluorescence was immediately measured. The standard curve was made of DCF in EtOH.

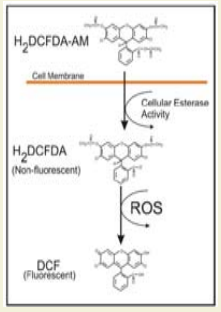

Fig 3: The acetylated form of DCHF-DA passes through the cell wall and enters the cytosol. The ester bond is cleaved, resulting in a pronated, hydrophilic molecule trapped in the cytosol

RESULTS

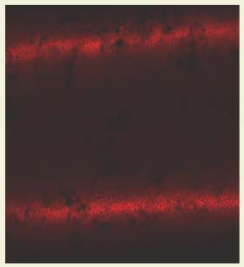

Fig 4: Cell that has not been exposed to DCHF-DA. Shows no fluorescence. Red color is auto-fluorescence from the chloroplasts

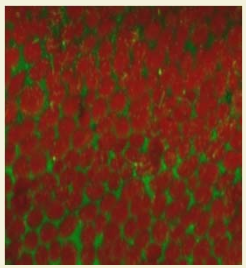

Fig 5: Cell that has been exposed to DCHF-DA, followed by a 1 minute rinse in $CaCl_2$/MES solution. No fluorescence is shown in the cell wall, but the green in the background indicates that the DCHF-DA has indeed passed through the cell wall and into the cytosol

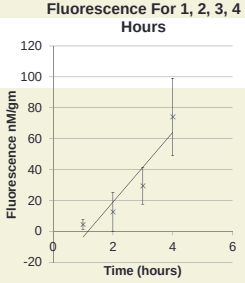

Fig 6: Time course ran to find optimal time for extraction. A linear relationship between fluorescence and time was established

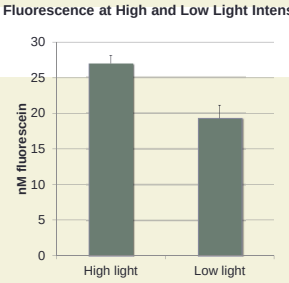

Fig 7: Time course ran to find optimal light settings for incubation. Low light was shown to minimize ROS produce in response to light

CONCLUSIONS

1. We have established that our technique for measuring ROS works. Through the confocal experiments, we have concluded that DCHF-DA does indeed cross through the cell wall and into the cytosol where it becomes trapped.
2. Through the time course experiments, we have also established that 2 hours is sufficient time for measuring fluorescence which indicates ROS production.

FUTURE/ ONGOING EXPERIMENTS

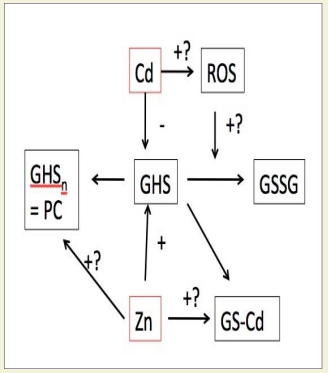

Fig 8: Possible pathways involving glutathione: reduced and oxidized glutathione, reactive oxygen species, metal-glutathione complexes, phytochelatins (PCs)

1) Discover the correlation between ROS production, cadmium uptake, and glutathione synthesis. Glutathione is the plants natural way of combating reactive oxygen species; it acts as an anti-oxidant which is important for stress management. It is a also precursor of phytochelatins, which chelate heavy metals like cadmium.

2) Plants will be kept in solutions of 2ppm cadmium, and also 2ppm cadmium and 5ppm zinc. ROS will be measured at various time periods to establish a correlation.

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