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Effects of Deuterium on *Chlamydomonas reinhardtii*

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ABSTRACT

In order to develop software tools to monitor rates of total protein turnover in plants, attempts were made to maximally label all the proteins in vivo. Our study focused on the use of the stable isotope of hydrogen, deuterium, to label proteins of *C. reinhardtii*. The goal of this study was to determine the effect of the label on algal growth. *C. reinhardtii* is a unicellular green algae that is heterotrophic, photoautotrophic and mixotrophic. The results showed that increasing concentrations of deuterium in the algal growth medium had a negative effect on the growth of the algae. These preliminary results indicated that other stable isotopes such as Nitrogen-15 and Carbon-13 should be assayed to see if, contrary to deuterium, they allow normal growth of the algae.

INTRODUCTION

The initial goal of this research is to develop protocols to achieve 100% labeling of total cell proteins so that the turnover rates of the total proteome can be determined. Full labeling of the plant proteome would facilitate the development of software that would make it possible to follow the changing proteome profile in response to environmental stresses. We have chosen to focus on proteins since they have critical roles in many processes including maintaining cell structure as well as serving as components in conveying signals to the nucleus and cytoplasm.

There are various techniques available for monitoring protein turnover including the use of stable isotopes (Pratt et al., 2002; Fedjaev et al., 2007). For this study we chose to use deuterium which is an isotope of hydrogen with a greater atomic mass due to one more neutron in the nucleus. This difference in neutrons allows deuterium-labelled peptides to be separated from unlabeled peptides by mass spectrometry (Burke and Mackay, 2008). Although deuterium is a simple isotope to use as it has no radioactive properties, Unno et al., (2003) reported that deuterium has a negative effect on yeast cell's growth. They reported that yeast cell lines grown in a deuterium medium had an increased expression of Hsp70 indicating higher stress levels than yeast cells grown in media prepared with water. Hsp70 is a chaperone protein that facilitates the maintenance and the folding of other proteins. For example, during heat stress, Hsp70 protects the cell by restoring the complex dynamitin/p50, which in turn prevents errors of replication during mitosis (Hut et al., 2005).

For this study we decided to look at the effects of deuterium on *Chlamydomonas reinhardtii* (Goodenough, 1992). If *C. reinhardtii* can grow in 100% deuterium without showing signs of stress then it would be easy to label and monitor protein turnover. *C. reinhardtii* is a motile, unicellular green algal cell with two flagella. It is a very useful organism in that it is photoautotrophic, heterotrophic, and mixotrophic; thus able to grow in different media and under different conditions. These characteristics of this model organism along with the fact that its genome was sequenced make *C. reinhardtii* ideal for our study. Growth of algae in medium prepared with heavy water resulted in reduced growth rate: the higher the deuterium concentration in the media, the less the algae grew. Thus we propose that deuterium is not a good label for our system as we are looking for a label that does not affect the organism.

MATERIALS AND METHODS

The strain of *C. reinhardtii* used in this study was CC-1266. The algae were grown in TAP medium (Tris Acetate Phosphate) (Harris, 1989) containing different concentrations of deuterium including 30%, 60%, and 90%. The deuterium was added by simply replacing the water used in the medium with the specific amount of deuterium. Algae were also grown in regular TAP medium in which none of the water was replaced for a control.

Different tubes containing 6 mL or 50 mL of media were shaken continuously under fluorescent light with a 16/8 hour photoperiod (Figure 1).

To measure algal growth absorbency readings were made once a day during lag phase and twice a day during the log phase. These readings were made using a spectrophotometer at a wavelength of 600 nm. To start the cultures, 1 mL of *C. reinhardtii* CC-1266 was added to 5 mL of medium, or in the case of the 50 mL cultures, 5 mL was added to 45 mL of medium.

RESULTS AND DICUSSIONS

As the concentration of deuterium was increased in the growth medium, the growth of the algae declined. This difference in growth is illustrated in Figure 2.

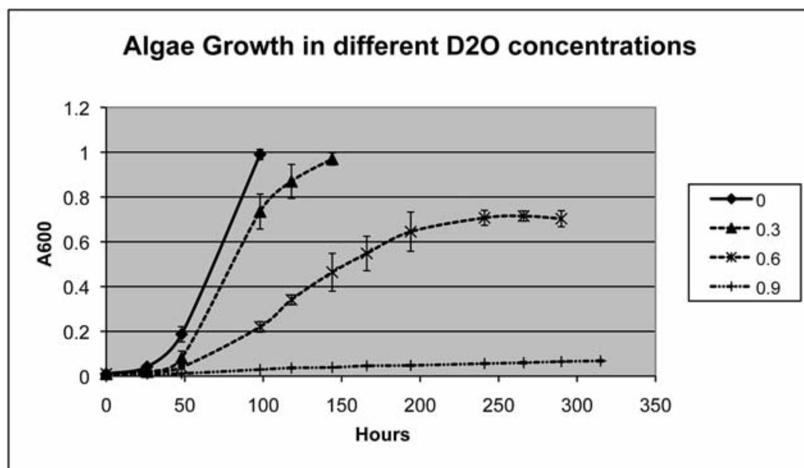

Figure 2. Effect of increasing deuterium concentrations on algal growth. Four different concentrations of deuterium were tested: 0%, 30%, 60%, and 90%. Growth readings were made using a spectrophotometer at 600 nm. Readings were taken once a day during lag phase and twice daily during log phase.

The effect of deuterium on algae can be caused by different factors. For example deuterium has been reported to have a negative effect on mitotic spindles as shown by studies on sea-urchin eggs and grasshopper spermatocytes (Lamprecht et al. 1991). They believed that deuterium blocks mitosis by either increasing or paralyzing the assembly of microtubules which delays the process. Also, Deuterium's heavy nature forces conformational changes in the structure of membrane proteins required for energy production (Vasilescu and Katona, 1986). This results in an imbalance of ADP/ATP and improper ATP production causing the destruction of ATP in the cell. The demand for more energy along with the suppressed ATP production makes for a weakened ATP reservoir and a tiring organism.

Significant morphological differences were also observed between cells grown in TAP and cells grown in TAP-deuterium as show in Figure 3.

Normal *C. reinhardtii* cells have a single big green chloroplast, two flagella, present a motile behavior and do not cluster. Algal cells grown in deuterium showed significant changes in cell characteristics. Those cells appeared less green, they would grow in clumps and didn't have flagellas (Figure 4). The cells that appeared clear had lost their chloroplast and were dead.

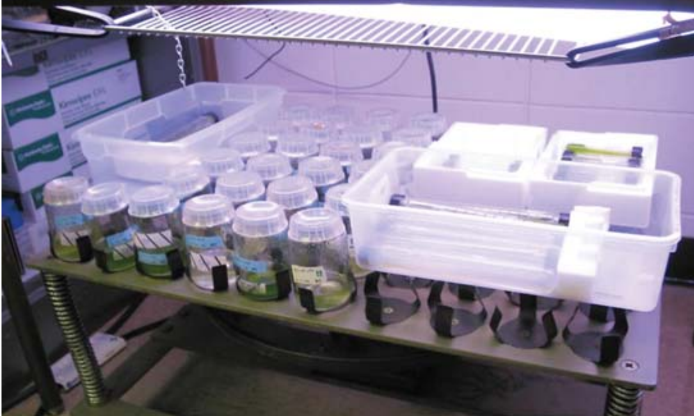

Figure 1. Algae cultures grown on a shaking table under fluorescent light. Cells were kept constantly shaking and on a 16/8 photoperiod. Different volumes of cells including 6 mL cultures and 50 mL cultures were grown.

Figure 3. Growth of CC-1266 in TAP media (left) and in TAP medium with 60% deuterium (right). We observed a clear change in color and the algae in deuterium appeared to form irregular clusters.

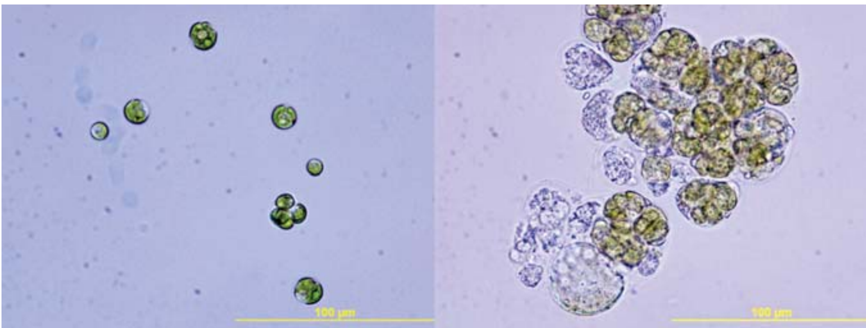

Figure 4. Microscopic evaluation of CC-1266 cells grown in TAP (left) and in TAP with 60% deuterium (right). Normal *C. reinhardtii* cells: green in color, motile, and two flagella. Deuterated *C. reinhardtii* cells: loss of color, irregular clusters, loss of flagella, and dead cells.

For many of these reasons we can conclude that using the stable isotope deuterium to label plant proteins cannot be our label of choice because we have shown that it significantly affects the growth and development of the algae. These preliminary results directed our research to using other stable isotopes including nitrogen-15 and carbon-13 to monitor protein turnover.

ACKNOWLEDGEMENTS

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