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REDUCED DEUTERIUM CONCENTRATION OF WATER STIMULATES O_2-UPTAKE AND ELECTROGENIC H^+-EFFLUX IN THE AQUATIC MACROPHYTE *ELODEA CANADENSIS*

Gábor LASKAY[§], Gábor SOMLYAI[&], Gábor JANCSÓ[#] and György JÁKLI[#]

[§]Department of Botany, University of Szeged, Egyetem u. 2., H-6722 Szeged, [&]HYD Ltd. for Research and Development, H-1539 Budapest POB 695, [#]KFKI, Atomic Energy Research Institute, H-1525 Budapest,POB 49, Hungary.

ABSTRACT: The effect of reduced deuterium concentration of water was studied on the rates of H^+-efflux out of and O_2-uptake by the cells of intact isolated leaves of the aquatic macrophyte *Elodea canadensis* by incubating the leaves in nutrient solutions prepared with distilled water having natural (150 ppm) and low (87 ppm) concentration of D in the presence and absence of light. In the presence of light, *Elodea* leaves in control water (150 ppm) brought about a gradual alkalinization of the external medium. In deuterium-depleted water, however, an external acidification was detectable in the first 30 minutes, the rate of which decreased later on, and after about 2 hours the rate of external alkalinization was comparable to that of the control. Associated fluorometric studies using the fluorescent probe 3,3'-dipropyl-thiacarbocyanine revealed an increase in the resting membrane potential (hyperpolarization), indicating that the observed external acidification can be associated with an increased activity of the H^+-ATPase in the plasma membrane. In addition, a stimulation of O_2-uptake in the dark was observed in the deuterium-depleted water. This effect exhibited a similar time-course to that of external acidification in the light. These observations demonstrate the ability of *Elodea* cells to detect and respond to a sudden reduction of the D-concentration of water. The response is transient in nature, and the physiological processes of the plant cell are capable of adapting to the altered conditions within several hours.

Keywords: deuterium, H^+-efflux, O_2-uptake, H/D isotope discrimination, *Elodea canadensis*

Corresponding author: G. Laskay, Department of Botany, University of Szeged, Egyetem u. 2., H-6722 Szeged, Hungary
Phone/Fax: +36-62-544-236
E-mail: glaskay@bio.u-szeged.hu

INTRODUCTION

The effect of increased D concentration on various physiological processes has been investigated extensively in different biological systems.[1,2] It was found that microorganisms as well as unicellular algae can be adapted to grow in D-enriched media, whereas cells of higher organisms have not proved adaptable to extremely high concentration of D.[2] Deuterium isotope effects on various enzyme reactions have been reported relatively early.[3,4] More recently, a marked inhibition of the plasma membrane H^+-ATPase in the presence of heavy water was observed in yeasts, indicating that the yeast H^+-ATPase can discriminate between H^+ and D^+, being unable to transport D^+ instead of H^+.[5]

Very little is known about the sensitivity of biological processes to reduced D concentrations. Somlyai et al.[6] were the first to demonstrate that sub-normal D concentration leads to the inhibition of cell division in cultured proliferating animal cells, indicating that naturally occuring D is essential for normal growth rate of cells. Practically nothing is known, however, about the sensitivity (if any) of plant cells to reduced D concentration of water. Aquatic plants, being in permanent contact with their external aqueous environment, are particularly suitable objects for studying the physiological effect brought about by any change in the composition of the external solution. We have attempted, therefore, to study the effect of sub-normal D concentration on several key parameters of cell physiology in an aquatic plant (*Elodea canadensis*). In this communication we report on definite, however transient, cellular responses.

MATERIALS AND METHODS

Whole plants of *Elodea canadensis* were cultivated in artificial pond water[7] supplemented with 1% Peretrix micronutrient solution containing 10% (w/v) N, 7% P, 5.5% K, 0.026% Mn, 0.003% B, 0.006% Cu, 0.002% Co, 0.008% Zn, 0.005% Mo, 0.02% Fe, 0.005% Mg (from Peremarton Chemical Co., Peremarton, Hungary), using 16/8 hour light/dark cycles at 22 °C. Intact leaves were detached and placed in nutrient solution

containing 1.0 mM NaCl, 0.1 mM K_2SO_4 and 0.5 mM $CaSO_4$ [8], prepared with distilled water having 150 ppm (control water) or 87 ppm D (deuterium-depleted water, Dd). Deuterium-depleted water was obtained by fractional distillation. The concentration of deuterium was determined by infra-red spectroscopy with a precision of ±3 ppm using a Foxboro Miran 1A CVF IR spectrophotometer (at 4 μm wavelength, in a 0.2 mm CaF_2 cell). The pH of the external solution was monitored by a combined pH-electrode connected to a Radelkis precision laboratory pH-meter (Radelkis Co., Hungary), and the O_2-concentration of the incubating solution was determined polarographically using a Clark-type O_2-electrode (Rank Brothers, Bottisham, England) equipped with a Hansatech O_2 electrode control box CB1D (Hansatech Ltd., King's Lynne, England). The changes in the membrane potential of the cells in intact leaves were monitored fluorometrically by the use of 3,3'-dipropyl-thiacarbocyanine (di-S-C_3-(3)), a potential-sensitive fluorescent probe. Labelling of the leaf cells was performed by the addition of di-S-C_3-(3) in 2 μM final concentration to the nutrient solutions and the fluorescense intensities were measured at excitation and emmision wavelengths of 540 and 590 nm, with 5 and 10 nm slits, respectively.

RESULTS AND DISCUSSION

Intact *Elodea* leaves in control water (150 ppm D) were found to alkalinize the external medium in the presence of light while leaves in Dd-water (87 ppm D) caused a transient acidification of the external medium (Fig. 1). The effect was maximal at around 30 minutes of exposure to Dd- water, later on the rate of acidification gradually slowed down and after about 2 hours the leaves appeared to cause 'normal' external alkalinization at a rate comparable with that of the control. This observation can be interpreted by assuming an increased activity of the H^+-ATPase present in the plasma membrane. To test this hypothesis, the resting membrane potential of the leaf cells during this period was monitored fluorometrically. These studies revealed an increased cell-associated fluorescence in Dd-water when the leaves were labelled with the fluorescent probe (Fig. 2).

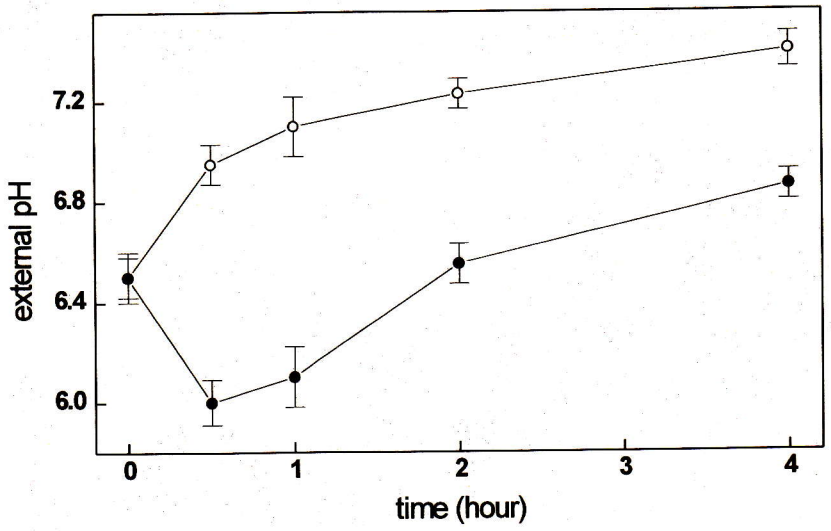

Fig. 1 Time-course of the pH-changes of the external nutrient solution, prepared with distilled water (40 cm^3) containing 150 ppm (○) or 87 ppm (●) D, caused by intact *Elodea* leaves (20 pieces) in the presence of light at 22 °C.

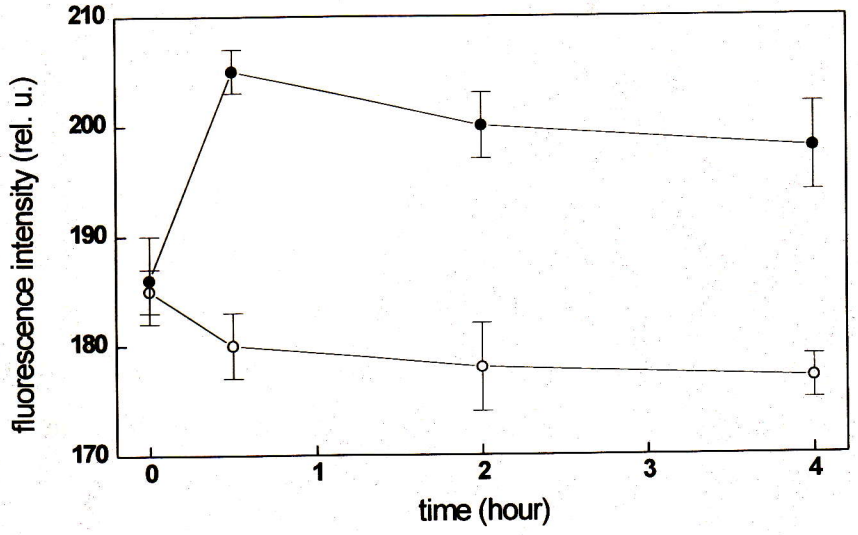

Fig. 2 Fluorescence intensity of intact *Elodea* leaves labelled with di-S-C_3-(3). Leaves were placed in nutrient solution, prepared with distilled water containing 150 ppm (○) or 87 ppm (●) D in the presence of light at 22 °C.

The di-S-C_3-(3) fluorescent probe accumulates in cells in proportion to the actual membrane potential, where its fluorescence quantum yield is increased.[9] By inference, our finding is indicative of a hyperpolarization occuring in the early phase of exposure of the leaves to Dd-water.

The consumption of O_2 in the dark was found to be stimulated when intact *Elodea* leaves were immersed in Dd-water - based nutrient solution, as compared to the control (Fig. 3). The stimulated O_2-uptake was detectable after as early as 15 minutes, increasing during the first 40-60 minutes, then it gradually declined and approached the control rate after 2-3 hours.

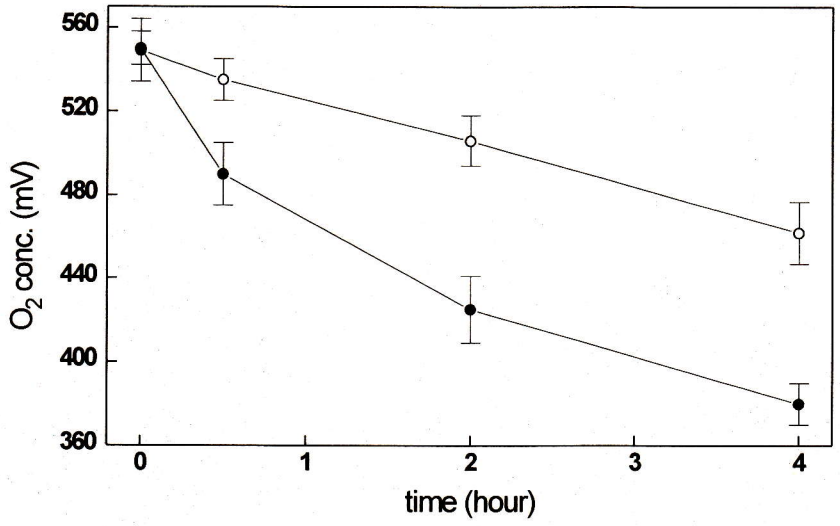

Fig. 3 Time-course of O_2-uptake by intact *Elodea* leaves (20 pieces) at 22 °C in the dark as a function of incubation time in nutrient solution prepared with distilled water (40 cm^3) containing 150 ppm (○) or 87 ppm (●) D.

These results clearly demonstrate that mature leaf cells of the aquatic macrophyte *Elodea canadensis* produce a well-defined response to a reduction of D concentration of the external solution, exhibiting a transient stimulation of H^+-efflux and of the rate of

O_2-uptake. Several biochemical processes, such as glycolysis, were found to be associated with hydrogen isotope discrimination.[10] Since glycolysis itself can be considered as the anaerobic part of the overall respiratory sequence, supplying the majority of respiration substrates (e.g. pyruvate), one can speculate on a possible connection between the D/H discrimination during glycolysis and the observed stimulation of O_2-uptake. On the other hand, mitochondrial processes *per se* have not been found to be involved in H/D isotope discrimination.[11] This suggests that the observed stimulation of respiration might be considered as a general response of the cells.

The observations presented in this communication demonstrate the ability of *Elodea* cells to detect and respond to a sudden reduction of the D-concentration of water. It is concluded that a sudden decrease of D concentration brings about an initial "shock", presumably beacause of the perturbation of the preexisting intracellular D/H ratios, evoking a response elicited by the cells. Importantly, however, the response is transient in nature, and the physiological processes of the plant cell are capable of adapting to the altered conditions within a few hours.

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