Phyton (Horn, Austria)	**Vol. 36**	Fasc. 2	295–302	31. 12. 1996

The Role of Calcium in the Stress Response of the Halotolerant Green Alga *Dunaliella bardawil* BEN-AMOTZ et AVRON

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With 1 figure

Received

Key words: *Dunaliella bardawil*, stress, Calcium, cell volume, ions uptake, glycerol.

Summary

ISSA A. A. 1996. The role of calcium in the stress response of the halotolerant green alga *Dunaliella bardawil* BEN-AMOTZ et AVRON. – Phyton (Horn, Austria) 36 (2): 295–302, 1 figure. – . English with German summary.

The effect of hypertonic shock on *D. bardawil* cells in the presence or absence of Ca^{2+} (1,5,10 mM) on cell volume and its metabolites were followed. When the Na^+ concentration of the medium was increased from 0.5 M to 4 M, the volume of packed *D. bardawil* cells shrank (2 min.), followed by cell volume-regulation phase; in which the cells swell (1–2 h). In the presence of Ca^{2+}, a slight recovery in the cell volume was observed by decreasing the uptake of ions (Na^+ & $C1^-$) in comparison to the cultures containing NaCl alone. Generally, Ca^{2+} has a positive role in the photosynthetic oxygen evolution and dark oxygen uptake in *Dunaliella* cells. Moreover, the intracellular glycerol contents of *D. bardawil* cells was sharply decreased whereas the carbohydrate content was increased by low concentrations of Ca^{2+} (1 & 5 mM).

Zusammenfassung

ISSA A. A. 1996. Die Rolle von Kalzium in der Reaktion auf Stress bei der salztoleranten Grünalge *Dunaliella bardawil* BEN-AMOTZ et AVRON. – Phyton (Horn, Austria) 36 (2): 295–302, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Der Effekt eines hypertonischen Schocks auf *D. bardawil*-Zellen wurde bei Vorhandensein oder Fehlen von Ca^{2+} (1, 5, 10, mM) im Hinblick auf Zellvolumen und Zellmetaboliten verfolgt. Wenn sich die Na^+-Konzentration des Mediums von 0,5 M

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auf 4 M erhöhte, schrumpfte das Volumen von zusammengepackten Zellen von *D. bardawil* (2 Minuten). Darauf folgte eine Phase der Zellvolumsregulation, in welcher die Zellen wieder anschwollen (1 bis 2 Stunden). In der Gegenwart von Ca^{2+} konnte, im Vergleich zu Kulturen, welche nur NaCl enthielten, eine leichte Wiederherstellung des Zellvolums beobachtet werden, indem die Aufnahme von Ionen Na^+ und Cl^-) vermindert wurde. Allgemein gesehen spielt Ca^{2+} bei der photosynthetischen Sauerstoffentwicklung und bei der Sauerstoffaufnahme im Dunkeln eine positive Rolle in den *Dunaliella*-Zellen. Darüberhinaus verminderte sich der intrazelluläre Gehalt an Glycerol bei *D. bardawil*-Zellen deutlich, wogegen der Kohlenhydratgehalt durch geringe Ca^{2+} Konzentrationen (1 und 5 mM) anstieg.

Introduction

Dunaliella is a genus of green unicellular algae characterized by the ability of tolerating higher salt concentrations than any other green plant. Many species of *Dunaliella* can live in salt solutions varying from dilute to saturated. Since the cells of this genus do not possess a rigid cell-wall, they respond to changes in salt concentration by rapid alterations in cell volume and then return to their original volume as a result of adjustments in the amounts of intracellular ions and glycerol, this latter being the major osmoticum (Borowitzka & Brown 1974, Ben-Amotz & al. 1982, Ginzburg 1987, Cowan & al. 1992 and Ginzburg & al. 1995). The halotolerant *Dunaliella bardawil* responds to osmotic stress by regulating the flux of carbon between synthesis of starch in the chloroplast and production of glycerol in the cytoplasm (Bental & al. 1990). Calcium is an important factor in the resistance of plants to salinity. Abdel-Basset 1993 found that, Ca^{2+} in certain ratios to Na^+ reversed most of NaCl stress symptoms in *Chlorella vulgaris*. Ca^{2+} functions as a general membrane stabilizer (Leopold & Willing 1984), alleviate injury caused by dehydration by stabilization of membrane system of cells as well as of subcellular organelles during water stress (Abdel-Basset & Issa 1994). The aim of this work was to follow the role of Ca^{2+} in the stress response of the halotolerant green alga *Dunaliella bardawil* as a function of time.

Materials and Methods

Algal material and culture conditions

Dunaliella bardawil Ben-Amotz et Avron was isolated from a salt pond near Bardawil lagoon, North Sinai, Egypt. Cells exhibited a strong red colour (red form). The red form was stepwise adapted during two months from its natural medium to seawater supplemented with 0.5 M NaCl, 8 mM KNO_3, 2 mM $MgSO_4$, 1.9 mM $MgCl_2$, 0.01 mM $Ca(NO_3)_2$, 4 mM K_2HPO_4 and micronutrients after Surzycki 1971. During this period the chlorophyll content increased and, as a consequence the alga took on a green colour (green form). The green form was grown at 26 $\pm$ 1 °C under continuous illumination with white light (175 µmol photons m^{-2} sec^{-1}). The cultures were transferred suddenly from 0.5 M NaCl to 4 M NaCl for 2 h with or without Ca^{2+}

concentration (1, 5 & 10 mM). The cell volume, ion uptake and some metabolites were followed (0, 30, 60, 90 & 120 min.) after transitions.

Analytical methods

Cell volume

The cell volume was estimated by measuring the length and width of *Dunaliella* with the aid of a microscope. The volume of cells was taken to be a revolution ellipsoid and calculated according to the formula $V = 4/3\ \pi\ ab^2$ (a taken as the half-length and b the half-width of the cell). The mean cell volume was calculated from measurements of 50 cells. Volume changes were expressed by the ratio V/V_o, where V_o is the average cell volume in isotonic medium and V the mean cell volume in anisotonic medium.

Na^+ and Cl^- contents

Two difficulties had to be overcome in order to measure the ion content of *Dunaliella* cells, contamination of the cell with NaCl from the highly saline outside medium and possible loss of ions from the cells. A washing technique using very rapid centrifugation was used (EHRENFELD & COUSIN 1982, GINZBURG 1987). Cell suspensions were centrifuged at 10000 g for 15 min. and the resulting cell pellets dispersed in 10 ml double-distilled water after thorough rinsing of the walls of the tubes. Cell debris was sedimented by centrifugation. Na^+ contents were determined with a flame photometer (WILLIAMS & TWINE 1960) and C1 contents were determined by a volumetric method (JACKSON 1960).

Photosynthetic O_2 evolution and dark respiration

O_2 evolution in the light and O_2-uptake in the dark were determined using a Clark electrode (Type E 16 WTW GmbH, Weilheim) in a 3 ml containing temperature constant acrylic glass cuvette, in which the algal suspension was agitated by a magnetic stirrer. The light intensity was (1.6×10^3 µmol photons $m^{-2}sec^{-1}$). Chlorophyll was extracted with absolute ethanol and quantified using the absorption coefficients given by WINTERMAN & DEMOTS 1965.

Total carbohydrate

For the determination of the total carbohydrate, the anthrone sulphuric acid method was used (FALES 1951).

Intracellular glycerol

This was analysed by means of chromotropic acid (LAMBERT & NEISCH 1950) on pellets of cells after centrifugation.

Statistical analysis

Differences in oxygen exchanges, carbohydrate contents and cellular glycerol were tested for statistical significance between treatments, using one-way analysis of variance of means of three replicates (Pc-State computer program).

Results and Discussion

The effects of transferring cells of several different *Dunaliella* species from low to higher salinity have been documented in several papers which examined cell volume changes, glycerol formation and photosynthesis

(BRÜGGEMANN & al. 1978, BROWN & BOROWITZKA 1979, KESSLY & BROWN 1981, GIMMLER & al. 1981, GILMOUR & al. 1982, SADKA & al. 1989, ISSA 1991). This paper is, however, the first to follow the role of calcium concentrations in the stress response of *D. bardawil* as a function of time.

When the Na^+ concentration of the medium was increased from 0.5 M to 4 M, the volume of packed *D. bardawil* cells shrank (Fig. 1). The new volume was observed 30 min. after the increase in outside Na^+, followed by cell volume-regulation phase in which the cells swell (1–2 h after transitions). In this respect, GIMMLER & al. 1977 observed that, after salinity changes, as a first step algae behave like osmometers according to the Boyle Van't Hoff law and a second step, cells tend to recover their original volume. A slight recovery was seen in *D. bardawil* cell volume which subjected to hypertonic shock in the presence of 1, 5 & 10 mM Ca^{2+} whatever the period after transition (Fig. 1). Ca^{2+} is an important factor in the resistance of plants to salinity (LAHYE & EPSTEIN 1971, AHMED & al. 1989).

As regards to ion uptake, *D. bardawil* cells were found to take up large amounts of Na^+ and Cl^- (0-30 min. after treatment). Due to readaptation a sharp decrease in ion content (Na^+ & Cl) after 30 min. from transitions was observed (Fig. 1). The same results were obtained by EHRENFELD & COUSIN 1982. However, in the presence of 1, 5 & 10 mM Ca^{2+}, the readaptation of *D. bardawil* cells was observed also after 30 min from transitions but the ion content (Na^+ & Cl^-) were lowered. In this respect, NAKAMURA & al. 1990, ABDEL-BASSET & ISSA 1994, ISSA & al. 1995 noticed, that selective permeability and ion uptake through membranes is regulated by calcium.

There are various levels at which effects of salt stress on oxygen exchange reactions can be assessed (Table 1). One of these is to measure rate immediately after transition (0–30 min.) and relate the response to magnitude of the stress (the oxygen evolution diminished and the dark respiration enhanced). Another is to observe the rate of recovery over an extended period (1–2 h) after stress (the oxygen evolution enhanced and the respiratory oxygen uptake diminished). BROWN & BOROWITZKA 1979 working on *Dunaliella sp.* observed that the photosynthetic oxygen evolution was raised with increasing salt concentrations whereas the dark respiration was diminished. In this work, photosynthesis & respiration have also been correlated with Ca^{2+}, in which the oxygen evolution was higher and the oxygen uptake was lowered in cultures containing 1 or 5 mM Ca^{2+} (Table 1). Thus, Ca^{2+} may probably repair the NaCl injured thylakoids. The results of either stimulation or inhibition were very clear immediately after transition (0–30 min.) to salinity stress. The same results were obtained by FERGUSON 1984, ABDEL-BASSET 1993.

Hypertonic shock, progressively increased the total carbohydrate contents of *D. bardawil* cells, whatever the period after transition.

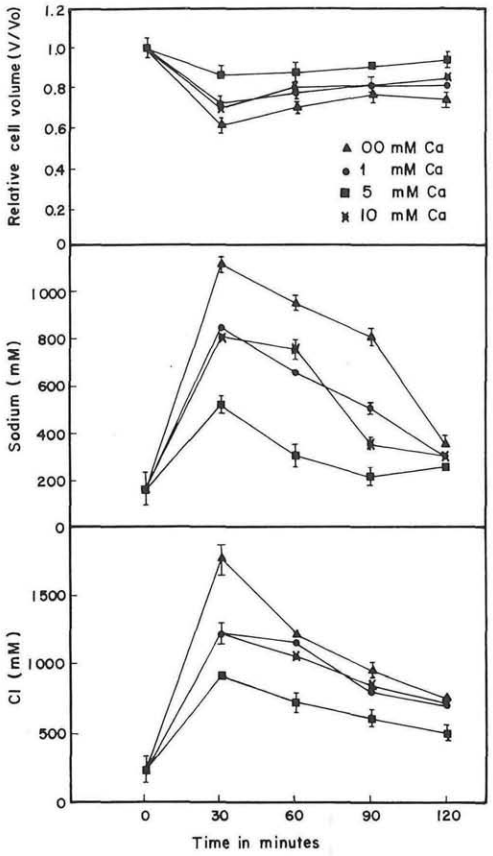

Fig. 1. The influence of osmotic hypertonic shock and role of calcium on relative cell volume (V/V_o) and cellular ions (Na^+ & Cl^-) of *Dunaliella bardawil* as a function of time. Values are means of three replicates.

However, in the presence of Ca^{2+} (1,5 mM) these contents were significantly decreased (Table 1). This is in agreement with the results obtained by GIMMLER & MÖLLER 1981.

A rapid osmoregulation in *D. bardawil* involves glycerol and starch metabolism; NaCl – stress direct the flow of fixed carbon towards glycerol synthesis at the expense of total carbohydrate formation (mainly starch). Many authors agree with this statement (HARD & GILMOUR 1992, GINZBURG & al. 1995). However, in the presence of Ca^{2+} the glycerol formation by *Dunaliella* were significantly lowered especially at 5 mM Ca^{2+} after 1–2 h from transition (Table 1). Similarly, AHMED & al. 1989 working with *Chlorella vulgaris* observed, that proline (the main organic osmoticum of

Table 1.

The influence of osmotic hypertonic shock and role of calcium on oxygen exchanges, carbohydrates and cellular glycerol of *Dunaliella bardawil* as a function of time.

Time (min)	0	30				60				90				120			
Ca^{2+} (mM)	0	0	1	5	10	0	1	5	10	0	1	5	10	0	1	5	10
Photosynthesis μ moles. O_2 mg chl-a.h^{-1})	28.6 cd	25.1 d	30.5 b	28.0 d	29.0 cd	29.8 bc	30.3 cd	29.0 cd	31.5 d	29.0 cd	28.2 cd	21.1 e	26.31 d	35.5 a	20.0 e	29.0 cd	23.6 d
Respiration μ moles. O_2 mg chl-a.h^{-1})	3.1 bc	3.90 b	3.1 bc	2.6 cd	3.4 b	3.1 bc	3.8 b	2.8 cd	3.4 b	3.5 b	3.3 a	2.3 cd	3.6 b	3.7 b	3.3 a	3.4 b	2.6 cd
Carbohydrates (mg. mg chl-a.$^{-1}$)	21.3 e	22.5 e	21.5 e	21.4 e	23.2 d	23.9 c	22.6 e	22.8 de	23.7 cd	24.2 bc	23.5 cd	24.5 b	24.5 b	26.0 a	25.0 b	24.2 bc	27.4 a
Glycerol μ moles. mg chl-a^{-1})	30.7 c	37.7 bc	33.3 bc	31.5 bc	33.1 bc	33.1 ab	38.5 ab	36.4 adc	38.6 ab	42.5 a	38.2 abc	37.4 abc	41.8 a	47.8 a	39.7 ab	37.8 abc	49.4 a

Each value represents the average of three replicates. Values followed by the same Letter(s) are not significantly different at 5% level by Duncan's multiple range.

Chlorella) accumulation was markedly enhanced in various salinized cultures, but in the presence of Ca^{2+} proline accumulation was retarded. Moreover, COWAN & al. 1992 stated that at least one of the proteins required for glycerol production in *Dunaliella salina*: Fructose 1,6-bisphosphatase, is a Ca^{2+}-dependent enzyme. It is worthy to point out, that the cultures having 10 mM Ca^{2+} do not recover and have a negative effect on the metabolites of *D. bardawil*. Finally, the potential controlling mechanism of Ca^{2+} in the stress response of *Dunaliella bardawil* deserves further investigation.

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