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Membrane stabilization and survival of dehydrated *Chlorella fusca* cells induced by calcium

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Abstract

Chlorella fusca was subjected to evaporative dehydration under air humidity of 72 %. Ca^{2+} pretreated cultures lost water as rapidly as untreated cultures. Nevertheless, an ameliorative effect of Ca^{2+} pretreatment in droughted cells was found as membrane stability index was improved and K^+ leakage was reduced. In addition, higher chlorophyll content and stability was observed. These parameters enabled droughted cells to recommence growth upon rewatering. Thus Ca^{2+} might increase survival of *C. fusca* cells subjected to drought through membrane stabilization.

Introduction

Drought induces numerous changes in plant cell structure and metabolism (for review see *e.g.* Levitt 1980). Membranes may play a central role as they participate in metabolic activities of plant either directly or indirectly (*e.g.* Rhodes 1987). According to Hubac *et al.* (1989), the integrity of cellular membrane structure and the preservation of membranes are of primary importance in the tolerance to water stress. Ca^{2+} functions as a general membrane stabilizer (Leopold and Willing 1984), prevents leakage of intracellular K^+ and support root cell elongation under salt stress (*e.g.* Nakamura *et al.* 1990). A certain Na^+/Ca^{2+} ratio was found to increase chlorophyll (Chl) contents in salt-stressed cultures of *Chlorella vulgaris* (Abdel-Basset 1986). Because algae are frequently subjected to drought in natural habitats, we studied effect of drought on membrane stabilization and survival of the unicellular green alga *C. fusca* and the possibility of amelioration of negative effects of drought by pretreatment with Ca^{2+}.

Materials and methods

Unialgal cultures of *C. fusca* were grown in batch cultures using Beijerinck's nutritive medium (Stein 1966) under continuous irradiation (5.9 W m^{-2}) and aeration

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at 25 ± 1 °C. This medium contains 0.09 mM Ca^{2+}. Aliquots of algal suspension were filtered through glass fiber filters (*Whatman* glass microfiber filters *GF/A*, constant mass, 50 mm diameter). The filters with algae were weighed and then exposed to air (relative humidity 72 %, temperature 20 °C) for 0, 2, 4, 8, 10, 12, 18 and 24 h. At each time the filters were reweighed for calculation of water loss. A known mass of the filter with adhering algae was submerged in 90 % acetone for Chl extraction and estimation according to Metzner *et al.* (1965). The stability of Chl was estimated after acidification by 1 M HCl using calculations of Lorenzen (1967). Another sample (filter) was submerged in deionized water, shaked for 3 h to get a cell suspension free from the filter, and then left at 10 °C for measuring membrane stability index (MSI). After 24 h, conductivity was measured and remeasured again after the sample was autoclaved using conductometer *YSI Model 35* (*Yellow Spring Instrument*, Yellow Spring, USA). MSI was measured as percentage of injury based on the procedure of Premachandra and Shimada (1988). K^+ leakage was estimated using flame photometry (Williams and Twine 1960) after submerging algal cells for 24 h in deionized water at 10 °C. The data of K^+ leakage were represented as mol kg^{-1}(d.m.) after substracting leakage at zero time. Growth rates of drought-stressed *Chlorella* cells were determined as changes in absorbance at 750 nm. Aliquots of cells freed from the filter were recultured for 3 d in fresh medium at the same conditions of growth. Ca^{2+} pretreatment was achieved by adding 0, 1, 5 and 10 mM Ca^{2+} ($CaCl_2$) to the algal suspension for 18 h. Aliquots of preloaded cultures were then filtered through glass fiber filters and the above procedures were applied, but at 0 and after 4 h of drought. The data are means of two reproducible replicates.

Results

Cells of *C. fusca* lost 40 % of water within 2 h at atmospheric humidity of 72 % and temperature of 20 °C) and after 4 h they lost 75 % of water. No further loss was recorded during prolonged evaporative dehydration for up to 24 h (Fig. 1). The addition of Ca^{2+} did not affect the amount of water lost, and the water loss after 4 h was 71 - 74 % of water content at zero time (Fig. 2). Membrane injury was increased at 2, 4 and 8 h. The MSI exhibited almost no more injury with longer time of desiccation, *i.e.* maximum injury was reached after 8 h (Fig. 1). Upon addition of Ca^{2+}, the level of injury was generally lowered, particularly at 1 mM Ca^{2+}. This treatment also improved MSI of non-droughted cells (0-time). Moreover, after 4 h of drought it relieved much of injury that was observed in droughted control cultures. The leakage of K^+ from droughted *C. fusca* cells, however, was continually increased along the 24 h of dehydration. Pretreatment with Ca^{2+} (10 mM) lowered K^+ leakage of dehydrated cells down to the level of the control cells at zero time (Fig. 3).

Contents of Chl *a*, Chl *b* and carotenoids were generally lowered by dehydration. However, after 2 h of evaporation, there was an increase in pigment contents (Table 1). Stability of Chl, as well, was concurrently lowered with time of drying (Fig. 1). About 50 % of Chl stability were lost after only 4 h of dehydration. However, in Ca^{2+} pretreated *C. fusca* cells, Chl content not only resisted dehydration

but even increased per unit dry mass (Table 2). Chl stability was also higher than that of control cultures (not loaded).

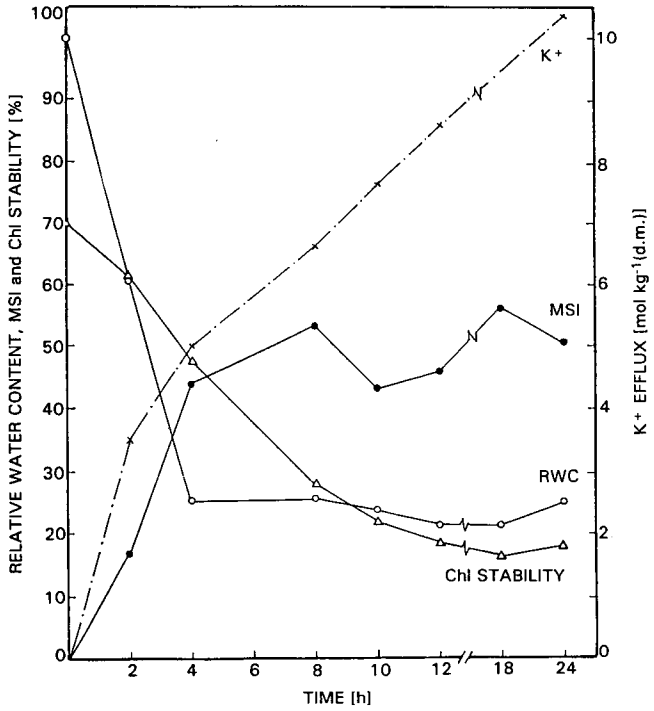

Fig. 1. Relative water content (RWC), membrane stability index (MSI), K^+ efflux and chlorophyll stability of *C. fusca* cells subjected to evaporative dehydration for 24 h.

Table 1. Photosynthetic pigments (chlorophylls and carotenoids) [g kg^{-1}(d.m.)] of *C. fusca* cells subjected to dehydration at relative humidity of air 72 %.

Time [h]	Chl *a*	Chl *b*	Car	Chl *a/b*	Chl *a+b*/Car
0	2.98	2.29	1.05	1.30	5.04
2	5.26	4.21	1.19	1.25	7.94
4	3.19	2.99	0.70	1.07	8.81
8	1.60	1.54	0.28	1.04	11.06
10	1.34	1.30	0.29	1.01	8.99
12	1.34	1.03	0.37	1.31	6.49
18	1.29	1.23	0.29	1.05	8.72
24	1.20	0.91	0.37	1.32	5.66

Growth recommenced in droughted *C. fusca* cells only after 2 h and to a lesser extent after 4 h *i.e.* when the cell water content decreased to 25 % of that at saturation. However, with similarly low water content, growth did not recommence because of long duration (8 - 24 h) of cells in dehydrated state (data not shown).

Ca^{2+}, however, greatly enhanced growth rates of dehydrated *C. fusca* cells (Fig. 4), despite it did not accelerate growth rates of water saturated cells (0-time).

Table 2. Photosynthetic pigments [g kg^{-1} (d.m.)] of *C. fusca* cells pretreated with Ca^{2+} and subjected to evaporative dehydration for 4 h.

Time [h]	Ca^{2+} [mM]	Chl *a*	Chl *b*	Car	Chl *a*/*b*	Chl *a*+*b*/Car
0	0	1.75	1.52	0.43	1.15	7.64
	1	1.45	1.09	0.45	1.33	5.68
	5	0.97	0.96	0.37	1.01	5.26
	10	1.17	1.14	0.42	1.03	5.47
4	0	1.53	1.37	0.34	1.12	8.58
	1	1.92	1.63	0.41	1.17	8.61
	5	2.06	1.71	0.53	1.20	7.08
	10	1.67	1.42	0.37	1.18	8.41

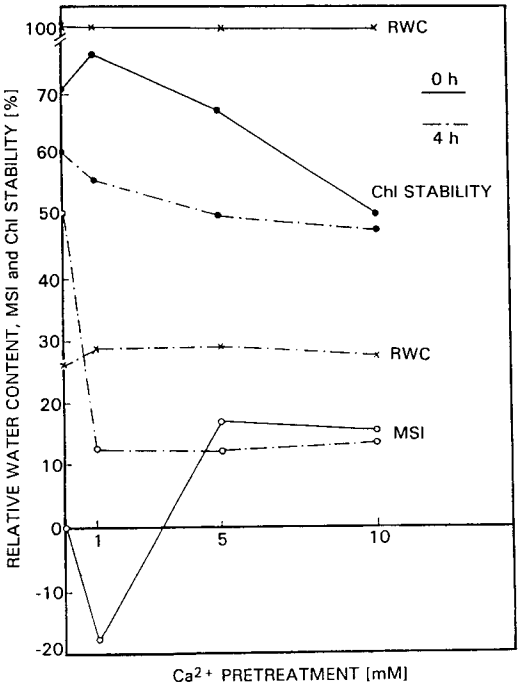

Fig. 2. Relative water content (RWC), membrane stability index (MSI) and chlorophyll stability of *C. fusca* cells pretreated with Ca^{2+} and subjected to evaporative dehydration for 0 or 4 h.

Discussion

Maximum water loss of *C. fusca* cells was attained rapidly after 4 h (75 %) at air humidity of 72 %. The addition of Ca^{2+} did not affect the amount of water lost.

Generally, none of the samples sustained after a period of only 4 h more than 30 % of water contained at saturation. The effect of Ca^{2+} may be due to ion-metabolism interaction rather than a water holding effect. Nakamura *et al.* (1990) concluded that external Ca^{2+} counteracts the ion-specific rather than the osmotic effects on mung bean roots. In *C. fusca* membrane stability index and leakage of K^+ could be correlated to drought. A correlation exists between the ion leakage and the drought

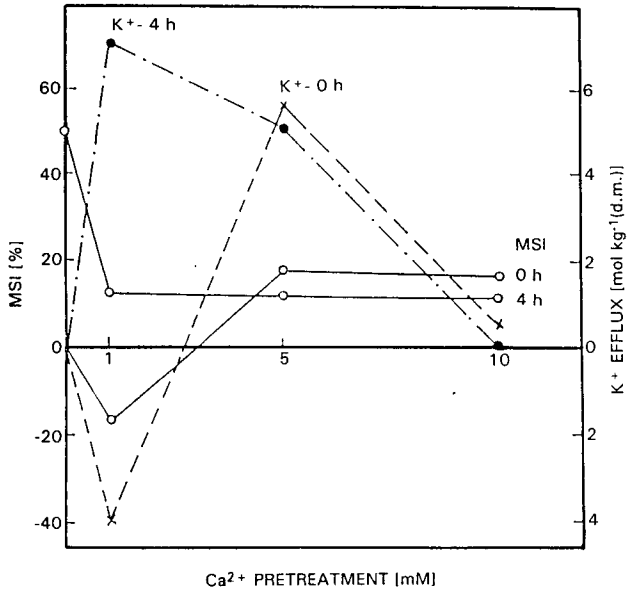

Fig. 3. Membrane stability index (MSI) and K^+ efflux from *C. fusca* cells pretreated with Ca^{2+} and subjected to evaporative dehydration for 0 or 4 h.

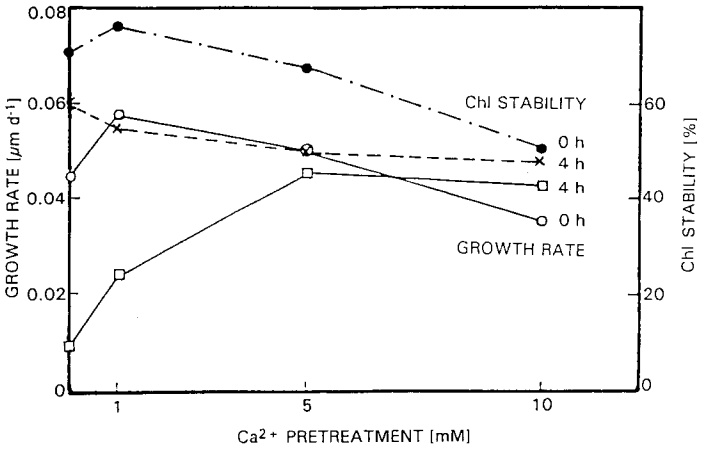

Fig. 4. Growth rate and chlorophyll stability of *C. fusca* cells pretreated with Ca^{2+} and subjected to evaporative dehydration for 0 or 4 h.

resistance (d'Aoust and Hubac 1986, Dassa-Girard 1987). Water or salinity stress injuries and tolerance are related to potassium efflux (Shcherbakova and Kacperska 1983, Nakamura *et al.* 1990). Upon addition of Ca^{2+}, the level of membrane injury as well as of K^+ efflux were generally lowered in dehydrated *C. fusca* cells. Similarly *Nakamura et al.* (1990) found that Ca^{2+} prevents the leakage of intracellular K^+ and thereby supports the elongation of roots under salt stress. Ca^{2+}, in addition, increased Chl contents per unit dry mass in droughted *C. fusca* cells. Similarly, Chl contents of salt stressed *C. vulgaris* increases at certain ratios of Na^+/Ca^{2+} (Abdel-Basset 1986). Not only Chl contents were increased by Ca^{2+} but also Chl stability. The recommence of growth by dehydrated *C. fusca* cells was also enhanced by Ca^{2+}. While control cells could not recommence growth after 2 h of dehydration, Ca^{2+} pretreatment enabled cells to regrow after 4 h of dehydration and at rates higher than those of cells not subjected to dehydration. Thus Ca^{2+} might alleviate injury caused by dehydration by stabilization of membrane system of cells as well as of subcellular organelles during drought. Subsequent decrease in leakage of ions and intermediate metabolites might be important for restarted growth. Under full hydration, however, Ca^{2+} might have a negative effect.

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