

Short Communication

Salt Adaptation of the Cyanobacterium *Synechococcus* 6311 Growing in a Continuous Culture (Turbidostat)

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ABSTRACT

Physiological aspects of salt-adaptation in the cyanobacterium *Synechococcus* 6311 growing in a continuous culture (turbidostat) were studied. The process of salt-adaptation was completed within 3 days, as expressed by the specific growth rate of cells grown in the presence of 0.2 and 0.4 molar NaCl. An increase in photosynthetic activity during the adaptation period leads to the accumulation of soluble sugars, essential for osmoregulation in the salt-grown cells. Cells grown in the presence of 0.4 molar NaCl showed an initial inhibition in the rate of protein synthesis which was enhanced after the 1st day of salt stress. After adaptation, salt-grown cells showed an increase in phycobiliprotein synthesis and a higher phycobiliprotein to protein ratio.

and illuminated with cool white fluorescent light, $I = 20 \text{ mw/cm}^2$.

Cell Parameters. Dry weight, Chl, protein, and phycobiliprotein content were determined as described previously (1). Total and soluble sugars were determined by the phenol-sulfuric acid method (5).

Cell Growth. Specific rate of growth and doubling time were calculated according to Van Liere *et al.* (9).

Oxygen Evolution. Oxygen evolution was measured with an Hansatech DW oxygen electrode. Different light intensities were obtained using plexiglass filters.

Rate of Synthesis. Rate of synthesis was calculated using the

Salt adaptation by fresh water cyanobacteria is composed of several mechanisms. Studies on the N_2 -fixing cyanobacterium *Nostoc muscorum* grown at high NaCl concentrations revealed stimulation of photosynthetic activity and sucrose accumulation (1), reorganization of the photosynthetic apparatus with changes in the thylakoidal organization (2), and the modification of the enzyme Fd-NADP reductase (7). Batch cultures of the non- N_2 -fixing cyanobacterium *Synechococcus* 6311 were shown to adapt to high NaCl concentrations, and the accumulation of soluble sugars (3) and K^+ ions was demonstrated (4).

This work describes physiological aspects of the process of salt adaptation in the turbidostat culture of the cyanobacterium *Synechococcus* 6311. Since the cell growth and composition are maintained constant in the turbidostat, the changes observed are a direct consequence of the salt-induced stress.

MATERIALS AND METHODS

Culture and Growth Conditions. *Synechococcus* 6311 was obtained from Prof. E. Padan, Hebrew University of Jerusalem. The cultures were grown routinely in a Kratz and Myers 'C' medium (6). Each of the cultures was originated from stock cells grown in Petri dishes with growth medium containing 1.5% agar. Two-week-old cultures were transferred to liquid medium, grown on a rotatory shaker in a temperature controlled room at $28 \pm 2^\circ\text{C}$, and illuminated with cool white fluorescent light, $I = 20 \text{ mw/cm}^2$. A continuous culture system (turbidostat) model CC-2 (Research and Development Authority, Ben Gurion University of the Negev, Israel) provided with glass chambers of 500 ml was utilized. The cultures were flushed with 3% CO_2 in air

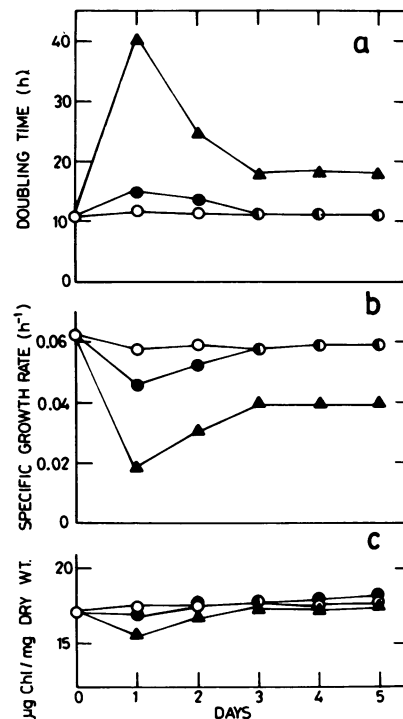


FIG. 1. Cell growth and Chl content in *Synechococcus* 6311 grown in the presence and absence of 0.2 and 0.4 M NaCl in a turbidostat culture. Cells were grown in a turbidostat as described in "Materials and Methods." NaCl was added after a constant specific growth rate was achieved. a, Doubling time of the culture; b, Specific growth rate; c, Chl content of the cells; (O), Control cells; (●), cells grown in the presence of 0.2 M NaCl; (▲), cells grown in the presence of 0.4 M NaCl.

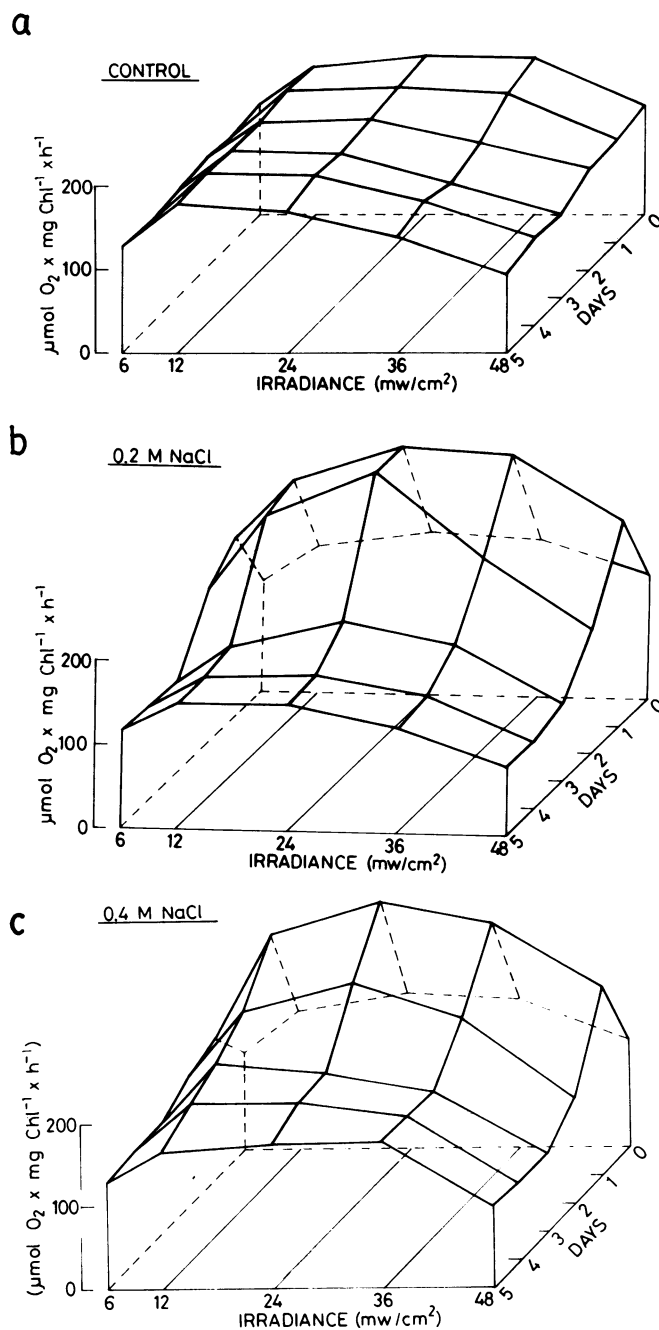


FIG. 2. The relationship between photosynthetic activity, light intensity, and growth time of *Synechococcus* 6311 cells. Cells were grown in a turbidostat as described in "Materials and Methods." a, Control cells; b, cells grown in the presence of 0.2 M NaCl; c, cells grown in the presence of 0.4 M NaCl.

formula:

$$R.S = (\Delta C \times V + C \times \Delta V) / T$$

where: $R.S$ = rate of synthesis

ΔC = change in cell content (mg/ml) during T

C = cell content (mg/ml)

ΔV = effluent during T (ml)

V = volume of the growth chamber = 500 ml

T = time (days).

RESULTS AND DISCUSSION

Salt adaptation was demonstrated in *Synechococcus* 6311 cells where NaCl at concentrations of 0.2 and 0.4 M was added to the

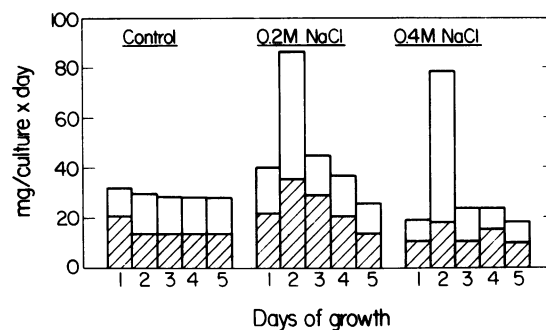


FIG. 3. Rate of synthesis of cell sugars in *Synechococcus* 6311 grown in the presence and absence of 0.2 and 0.4 M NaCl. Cells were grown in a turbidostat as described in "Materials and Methods." (□), Total sugar; (▨), soluble sugars.

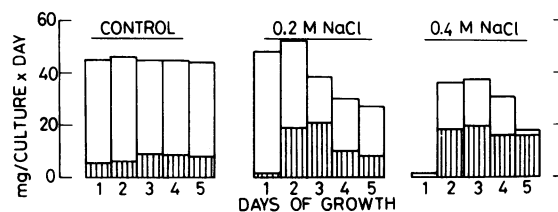


FIG. 4. Rate of synthesis of cell proteins in *Synechococcus* 6311 grown in the presence and absence of 0.2 and 0.4 M NaCl. Cells were grown in a turbidostat as described in "Materials and Methods." (□), Total protein; (▨), phycobiliproteins.

growth medium of the continuous culture. As the turbidity of the culture was maintained constant, the comparative study of photosynthetic activity and cell composition reflects the changes resulting from the different salt concentrations. Cells of *N. muscorum* were found recently to adapt to salt, but the degree of salt adaptation was determined by N_2 -fixing activity which is a more salt-sensitive process than photosynthetic activity (2). The cells of *Synechococcus* 6311 assimilate nitrate, and the activities of nitrate uptake and assimilation are less salt sensitive and provide great potential for salt adaptation.

Three identical cultures of *Synechococcus* 6311 cells were set in the turbidostat until a constant growth rate was obtained (specific growth rate = $0.06 \pm 0.004 \text{ h}^{-1}$), and NaCl was added to a final concentration of 0.2 and 0.4 M. Cell growth and composition were followed for 5 d.

The introduction of salt caused limited inhibition on the specific growth rate of cells grown in the presence of 0.2 M NaCl, and a marked inhibition in the cells grown in the presence of 0.4 M NaCl (Fig. 1b). The salt-stressed cultures recovered and adapted to salt within 3 d as indicated by the constant rate of growth. The doubling time of cells grown in the presence of 0.2 M NaCl was identical to that of control cells, while cells grown in the presence of 0.4 M NaCl displayed an increased doubling time after adaptation (Fig. 1a).

The Chl content was essentially stable throughout the process of salt adaptation (Fig. 1c). Cell volume was not affected significantly throughout salt adaptation of *Synechococcus* 6311 grown in batch cultures (results not shown).

The photosynthetic activity of *Synechococcus* 6311 control cells was relatively stable over a broad range of light intensities (Fig. 2a). The profile of photosynthetic activity was very different in the cultures grown in the presence of NaCl (Fig. 2, b and c). The rate of O_2 evolution was markedly enhanced during the first 2 d after exposure to salt, and was found stable for the following 3 d.

Increased photosynthetic activity during salt adaptation leads to the accumulation of sugars. The rate of synthesis of soluble

sugars in salt-grown cells was enhanced throughout the 5 d of growth (Fig. 3). The cells grown in the presence of 0.2 and 0.4 M NaCl continue to synthesize sugar during the enhanced photosynthetic activity of the first 2 d. The enhanced rate of carbohydrate synthesis in salt-grown cells provided the soluble sugars needed for osmoregulation. Sucrose and, to a lesser extent, glucose were shown to be the major components accumulated (3).

The enhanced photosynthetic activity was essential to provide the energy demand for the process of salt adaptation which includes the biosynthesis of sugars for osmoregulation and efficient Na⁺ exclusion (4). The enhancement in photosynthetic activity observed during the 1st d of adaptation is only partially utilized for soluble sugar accumulation, and may provide the energetic demand for active Na⁺ extrusion (8).

The enhanced photosynthetic activity in the salt-adapted cells during the first 2 d of growth was accompanied by an enhancement of the rate of phycobiliprotein synthesis; the phycobiliprotein to total protein ratio is very high in salt-grown cells (Fig. 4). The enhanced rate of phycobiliprotein synthesis continued for the 3 d following the leveling of the photosynthetic activity. Cultures grown in the presence of 0.4 M NaCl displayed an initial inhibition in the rate of total protein synthesis which was enhanced after the 1st d of salt stress. It is still unclear whether the newly synthesized phycobiliproteins are involved in protective mechanism in addition to their role in the light-harvesting proc-

ess.

In conclusion, cells of *Synechococcus* 6311 were shown to adapt efficiently to the salt stress applied in the turbidostat growth regime. The salt stress challenges the energy requirement for the biosynthesis of soluble sugars, necessary for osmoregulation, and for active Na⁺ extrusion (4).

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