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The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress

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Abstract. The responses of the filamentous cyanobacterium *Spirulina platensis* to increased NaCl concentrations (0.25–1.0 M) in addition to the concentration of sodium in the growth medium were studied. A two stage response to the salt stress was observed. This consisted of a relatively short shock stage, followed by adaptation process. It was shown that upon exposure to high salt concentrations of 0.5 M and above, immediate inhibition of photosynthesis and respiration, and complete cessation of growth occurred. After a time lag, the energy-yielding processes exhibited restored activity. At 0.5 and 1.0 M NaCl photosynthesis reached 80% and 50% that of the control, while respiration was enhanced by 140 and 200%, respectively. The time lags were longer when the cells were exposed to higher NaCl concentrations. The resumption of growth and the establishment of new steady state growth rates were found to be correlated to the recovery in respiration. The relationship between the growth rates after adaptation and the increased NaCl concentrations was found to be inversely linear. The cellular sodium content was maintained at a constant low level, regardless of the external NaCl concentration, while potassium content declined linearly vs. the external NaCl concentration. The carbohydrate content of the cells rose exponentially with the increase in NaCl concentration.

Key words: Cyanobacterium – *Spirulina* – Growth – Stress – Photosynthesis – Respiration – NaCl, Na^+, K^+

Spirulina platensis, a filamentous cyanobacterium has been isolated from a wide range of habitats differing in their water quality, from low ionic concentration through brackish to saline (Cifferi 1983). In some salty and high alkaline aqueous environments, *Spirulina* strains may form, at a given time, a bloom representing more than 90% of the total phytoplankton biomass (Richmond 1988). In recent years, considerable interest has been expressed in the outdoor cultivation of *Spirulina* biomass for commercial purposes (Vonshak and Richmond 1988; Richmond 1988). In such cultures growing under arid and semi-arid conditions, a daily evaporation rate of 1–2 cm was measured, leading to a constant increase in the salt concentration (Vonshak 1987).

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It is generally accepted that high intracellular sodium concentrations are toxic to most biological systems (Wyn-Jones and Gorham 1983). Adaptation to salinity requires development of mechanisms that limit the accumulation of sodium inside the cell. These mechanisms may consist of low permeability of the plasma membrane to sodium, combined with energy-consuming processes to extrude the entering sodium ions. Such a mechanism is the Na^+/H^+ antiporter in which the extrusion of sodium from the cell is coupled to the inwardly movement of the protons (Blumwald et al. 1984b; Krulwich 1986). Another aspect of adaptation to salinity is a build-up of internal organic osmotica in order to cope with the unbalanced osmotic pressure (McKay et al. 1984; Reed 1986; Hagemann et al. 1987). Recently, Warr et al. (1985) have shown that in *S. platensis* osmotic adjustment, as a response to salinity, was achieved by intracellular accumulation of the carbohydrates glucosyl-glycerol and trehalose.

In this work, we studied the response of *S. platensis* to high NaCl concentrations by following the changes in growth, photosynthesis, respiration and the intracellular content of ions and total sugars. The interrelationships between these processes in the adaptation of *S. platensis* to salt stress are discussed.

Materials and methods

Organism and growth conditions. The filamentous cyanobacterium *Spirulina platensis* originally isolated from Lake Chad and obtained from Prof. C. J. Soeder (KFA Jülich, FRG) was cultivated in batch culture under sterile conditions in Zarouk's medium, pH 9.0 (Vonshak 1986). The sodium content of the medium was 250 mM, most of it as sodium bicarbonate. The flasks were kept on a gyrotory shaker at 30°C and continuously illuminated with cool-white fluorescent lamps providing 80 μmol photons $m^{-2} \cdot s^{-1}$ at the surface of the flasks.

NaCl stress. Exponentially growing cells were harvested and resuspended at a concentration of 1 μg chlorophyll ml^{-1} in fresh medium containing NaCl at the indicated concentrations (not inclusive of Na^+ already present in the medium). Growth was measured by following the increase in chlorophyll content or the change in absorbance at 560 nm of the culture. Chlorophyll was assayed as described by Bennet and Bogorad (1973). Photosynthesis was assayed as

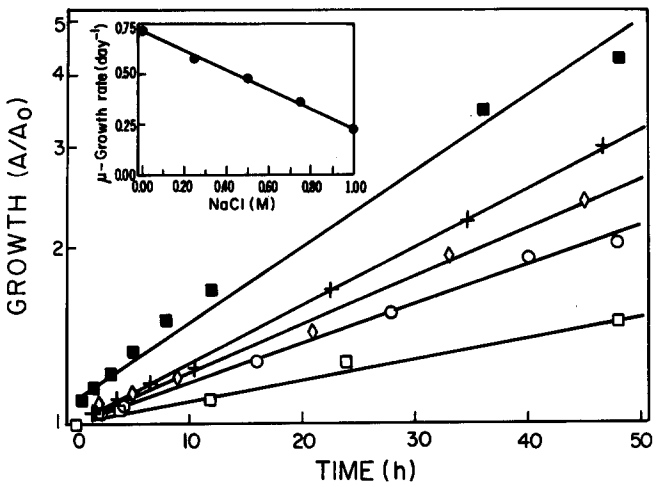

Fig. 1. Effect of NaCl on growth of *S. platensis* after adaptation to increased salt concentrations. A_0, absorbance at time zero; A, absorbance at any given time. Each point represents an average of 5 experiments. ■——■ control, +——+ 0.25 M, ◇——◇ 0.5 M, ○——○ 0.75 M, □——□ 1.0 M. Inset: Specific growth rates as a function of NaCl concentration

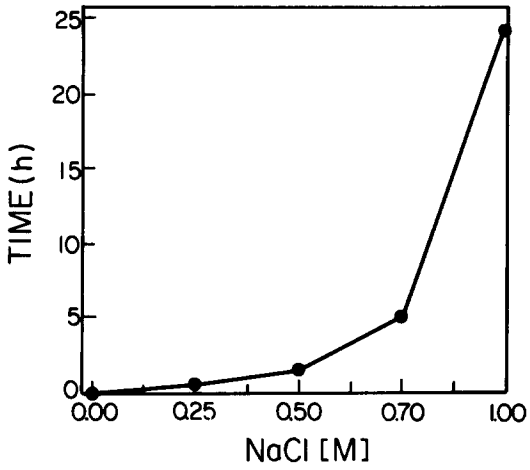

Fig. 2. Effect of NaCl concentration on the time lag before new exponential growth of *S. platensis* commenced. Each point represents an average of 5 experiments

the light-dependent O_2 evolution by means of a Clark type oxygen electrode (Yellow Springs, OH, USA). The chlorophyll concentration in the measuring chamber was 2–2.5 μg ml^{-1}, the temperature was 30° C and illumination was provided by a slide projector lamp at an intensity of 700 μmol photons $m^{-2} \cdot s^{-1}$. Dark respiration was measured as O_2 uptake.

Determination of sodium, potassium and carbohydrates. Cells grown at different NaCl concentrations were harvested by vacuum filtration, washed with two volumes of cold growth medium, in which sucrose (0.3 M) replaced the sodium and potassium ions, and then oven dried. The dried matter was resuspended and homogenized in 15 mM lithum solution (internal standard concentrate – Corning, NY, USA) to a final concentration of 2 mg dry matter ml^{-1}. Sodium and potassium were determined simultaneously in a Corning 435 flame photometer. Cell volume was measured as the fraction of the volume of the suspension. Five milliliters of cells were centrifuged to constant packing in a graduated hematocrit type test tube, and the packed cell volume was measured. Total sugar determination was performed by hydrolyzing the carbohydrates of the cell suspension with HCl, and assaying the sugar content by the anthrone method (Hassid and Abraham 1957).

Results and discussion

Exposure of *Spirulina* cultures to increasing NaCl concentrations resulted in an immediate cessation of growth. After a time lag a new steady state of growth was established. The new exponentially growth rates of the cultures grown in the various NaCl concentrations are shown in Fig. 1. These new growth rates were slower and were inversely correlated to the increased NaCl concentration in the medium (Fig. 1 inset). A decrease in the growth rate due to salt stress has been demonstrated in other cyanobacteria, such as *Anacystis* (Vonshak and Richmond 1981) and *Nostoc* (Blumwald and Tel-Or 1982).

For each salt concentration a different time lag was observed before growth commenced (Fig. 2). The length of the time lag was exponentially correlated to the degree of stress imposed on the cells (Fig. 2).

It has been suggested that exposure to high salinity is accompanied by a higher demand for energy by the affected cells (Blumwald et al. 1984a). We therefore thought it of interest to investigate the effect of salinity on the main energy producing processes – photosynthesis and respiration. The changes in photosynthetic and respiratory activity were followed over a period of 30 min to 48 h, after exposure to 0.5 and 1 M NaCl and were compared with the changes in biomass concentration as an indicator of growth (Fig. 3). A marked decrease in photosynthetic oxygen evolution was measured 30 min after exposure to the salt at both concentrations (Fig. 3a). This decline was followed by a recovery period which was characterized by a lower steady state rate of photosynthesis. Recovery at 0.5 M NaCl was faster than at 1.0 M NaCl (after 1.5 h vs. 3.0 h) and levelled off at 80% of the control activity vs. about 50% respectively. Respiratory activity also dropped rapidly immediately after the salt application at both concentrations (Fig. 3b). Activity was restored ten times faster at 0.5 M than at 1.0 M NaCl and continued to rise up to twice the control level at 1.0 M NaCl).

The immediate inhibition of the photosynthetic and respiratory systems after exposure to salt stress can be explained according to Ehrenfeld and Cousin (1984) and Reed et al. (1985) who showed a short-term increase in the cellular sodium concentration due to a transient increase in the permeability of the plasma membrane during the first seconds of exposure to high salt. It has been suggested that the inhibition of photosynthesis due to the rapid entry of sodium, might be the result of the detachment of phycobilisomes from the thylakoid membranes (Blumwald et al. 1984a). Elevated activities of dark respiration in cyanobacteria due to salinity stress have previously been reported (Vonshak and Richmond 1981; Fry et al. 1986; Molitor et al. 1986). The possible relevance of this phenomenon to salt adaptation is discussed later.

The relationship between the cellular content of sodium and potassium to the external concentration of NaCl is

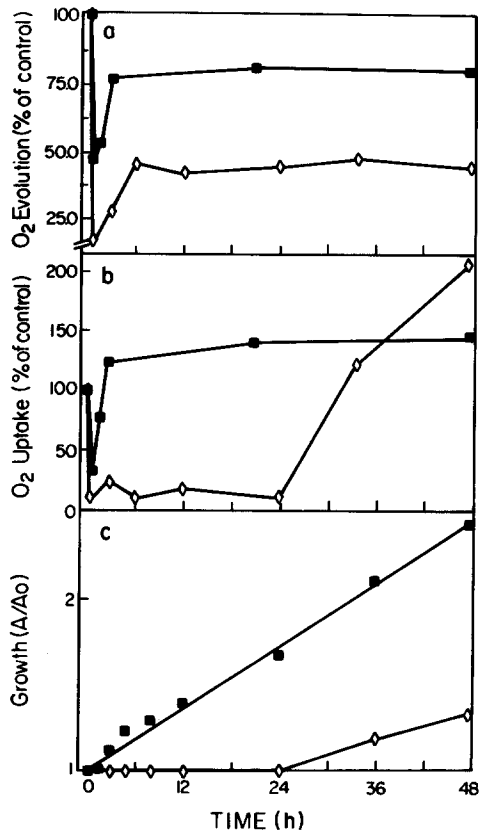

Fig. 3. Effect of NaCl on photosynthesis (**a**), respiration (**b**) and growth (**c**) in *S. platensis* exposed to ■——■ 0.5 M or ◇——◇ 1.0 M NaCl. 100% activity for apparent photosynthesis and respiration were 663 and 70 μmol O_2 · mg chl^{-1} · h^{-1}, respectively. Each point represents an average of 3 experiments

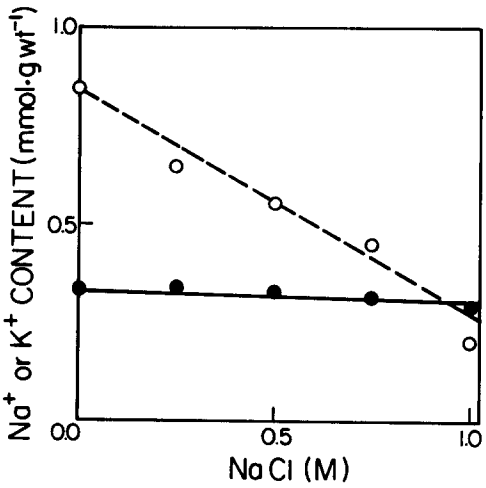

Fig. 4. Effect of NaCl concentration on the cellular content of Na^+ ●——● and K^+ ○——○ in *S. platensis* after adaptation to salt stress. Each point represents an average of 3 experiments

shown in Fig. 4. The data shown were obtained from cultures grown at their new steady state for 2–3 days. The amount of sodium was nearly constant over the entire NaCl concentration range, while a fourfold decline in the potassium content at 1.0 M was observed. This corresponds to an estimated cellular concentration of 30 mM for sodium and

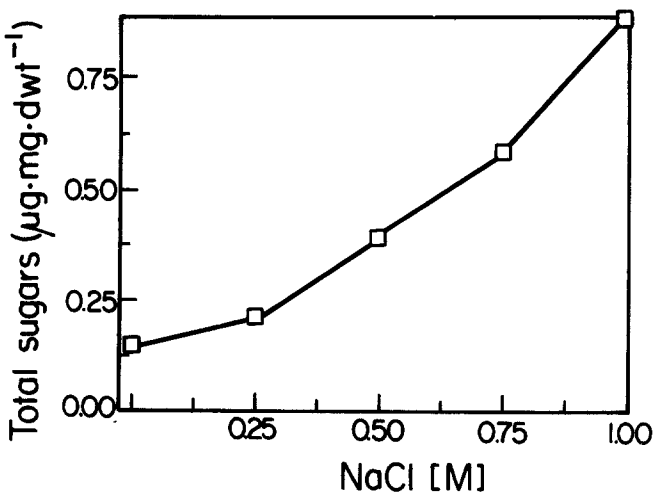

Fig. 5. Effect of NaCl concentration on the cellular content of total sugars in *S. platensis* after adaptation to salt stress. Each point represents an average of 3 experiments

90 mM for potassium before the exposure to salt, and 45 mM potassium for cells exposed to 1.0 M NaCl. These values are probably an over-estimation as no correction for the excluded volume was made. The results indicate that in *S. platensis*, sodium and potassium do not serve as a major osmotica in the cells, and that in spite of a large, inwardly directed sodium electrochemical potential gradient, the cells maintained a low internal concentration of this ion. Similar values of internal sodium concentrations were reported for the cyanobacterium *Synechococcus* 6311 (Blumwald et al. 1984b) and for the halotolerant alga *Dunaliella* (Katz and Avron 1985).

An exponential increase of the total sugar content was found in the cells grown under salt stress (Fig. 5), indicating that in this particular strain of *S. platensis*, carbohydrates are the major osmotica.

In conclusion, it was demonstrated in this work that the response of the cyanobacterium *S. platensis* to high salinity consisted of two stages. The first stage is characterized by an immediate inhibition of photosynthesis and respiration and a complete cessation of growth.

In the second stage, the cells resume growth at high NaCl concentrations after an appropriate time lag. The fact that the sodium content of the cells was low regardless of the external sodium concentration indicates the existance of efficient mechanisms to extrude sodium ions from the cells. The exponential relationship found between external NaCl concentrations, the lag time in growth (Fig. 2) and total sugar content (Fig. 5) together with the fact that the degree of increase in respiration was greater at higher NaCl concentrations (Fig. 3b) and that its time lag of recovery from the initial shock, coincided with the time lag in growth, may point out to a functional relationship between these processes. Molitor et al. (1986) found that the increase in respiration in *Anacystis nidulans* grown in NaCl-enriched medium was due to the enhanced activity of the plasma membrane cytochrome oxidase. They suggested a functional link between this enzyme and the Na^+/H^+ antiporter. In the light of their suggestion, the enhancement in respiration that we found might have a dual purpose – a partial compensation for the reduced level of photosynthesis in the stressed cells, and a direct role in pumping out sodium ions. If indeed

growth in *S. platensis* requires a low internal sodium concentration, then the dependency of the onset of growth on the enhancement of respiration could be understood.

The slowed growth rates established by the adapted cells can be attributed to an energy shortage. In the stressed cells there is a higher demand for energy for pumping out the entering sodium ions and for the massive synthesis of carbohydrates as osmotica.

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