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Short Communication

Photosynthetic and Respiratory Activity in *Anacystis nidulans* Adapted to Osmotic Stress

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ABSTRACT

***Anacystis nidulans* cultures growing photoautotrophically in a minimal medium were exposed to different concentrations of NaCl, up to 0.4 molar. Initially, a marked decrease in photosynthetic activity took place, which was in direct relation to the salt concentration and which was not associated with a change in endogenous respiratory activity. After a period of exposure to salinity, a process of adaptation became apparent, being manifested in a partial reversal of the decline in photosynthesis and a marked increase in endogenous respiration.**

The changes in photosynthetic and endogenous respiratory activity in *Anacystis nidulans* in response to osmotic stress have previously been studied after a short exposure to increased osmoticum (2, 5). The present study is aimed at elucidating the modifications in the photosynthetic and endogenous respiratory activity in *A. nidulans*, first when exposed to salinity for a prolonged period during which adaptation occurs and then during recovery from the osmotic stress. Osmotic stress may be defined as an increase in the osmotic potential of the medium above the optimal for growth.

MATERIALS AND METHODS

A. nidulans strain No. 625, from the culture collection at Indiana University, Bloomington, was grown photoautotrophically in a minimal medium (4). The flasks were aerated with an air-CO_2 mixture (97:3, v/v) at 35 C and illuminated by four 20-w cool white fluorescent lamps. Cultures of *A. nidulans* were diluted twice a day in order to maintain them in a steady state at the logarithmic phase of growth. Cells were harvested by centrifugation and resuspended at a concentration of 2.5 to 3.0 mg Chl/l in fresh medium containing 10 mM sodium carbonate and different concentrations of NaCl. After incubation in dim light for 30 min, the cells were placed in an illuminated chamber, where the temperature was kept constant (30 C) and the rate of O_2 evolution in light and its uptake in the dark were measured by a Clark-type O_2 electrode (Yellow Springs, OH). During all experiments, the cultures were maintained axenic.

RESULTS AND DISCUSSION

A short exposure (30 min) of *A. nidulans* to increased concentration of NaCl resulted in decreased photosynthetic activity in proportion to the salt concentration (Table I). In contrast, NaCl had no effect on endogenous respiration in the dark (Table I). It seems either that endogenous respiration is more tolerant to salt than is photosynthesis or that it takes place in a site better protected from the influx of salt molecules.

Cells exposed for a prolonged period to concentrations of up to 0.4 M NaCl were able to reach a steady state of growth. The specific growth rate declined in correlation with the increase in the concentration, *i.e.* the higher the NaCl concentration, the lower the specific growth rate at steady state (Fig. 1). NaCl concentrations of more than 0.4 M caused a marked decrease in the Chl content of the cells, and death followed shortly afterwards.

Table I. *Photosynthetic and Endogenous Respiratory Activity in A. nidulans Undergoing Osmotic Shock*

Cells grown under control conditions (no NaCl added) were harvested and resuspended in fresh growth medium containing different concentrations of NaCl. O_2 evolution and uptake were measured after 30 min of incubation in NaCl.

NaCl Concentration	O_2 Evolution	O_2 Uptake
M	$\mu mol \cdot h^{-1}\ mg^{-1}$ Chl *a*	$\mu mol \cdot h^{-1}\ mg^{-1}$ protein
0.0	440	3.0
0.1	395	3.3
0.2	350	3.0
0.3	300	3.3
0.4	140	3.2

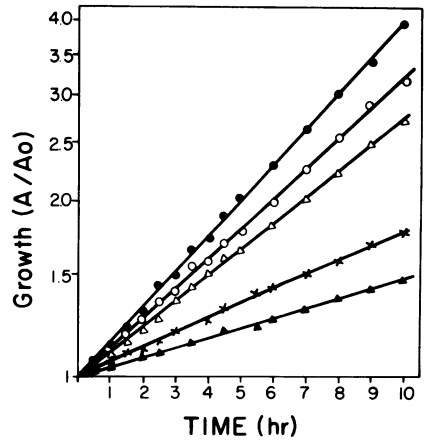

FIG. 1. Steady state growth rates of *A. nidulans* at different concentrations of NaCl. *A. nidulans* cells were grown at different concentrations of NaCl, and growth was recorded after steady state of growth was obtained. (●), Control; (○), 0.1 M NaCl; (△), 0.2 M NaCl; (×), 0.3 M NaCl; (▲), 0.4 M NaCl.

Table II. *Photosynthetic and Endogenous Respiratory Activity of A. nidulans Grown in Different NaCl Concentrations after Reaching Steady State*

A. nidulans cells grown at different concentrations of NaCl were harvested after a steady state of growth had been attained. Before the measurements, the cells were resuspended in fresh medium containing the same NaCl concentration as had the growth medium.

NaCl Concentration	O_2 Evolution	O_2 Uptake
M	$\mu mol \cdot h^{-1}\ mg^{-1}$ Chl *a*	$\mu mol \cdot h^{-1}\ mg^{-1}$ protein
0.0	440	3.0
0.1	414	3.9
0.2	377	4.5
0.3	380	4.8
0.4	216	6.0

Table III. *Photosynthetic and Endogenous Respiratory Activity in A. nidulans Grown at Various NaCl Concentrations Immediately after their Transfer to NaCl-Free Medium*

A. nidulans cells grown at steady state at different concentrations of NaCl were harvested and resuspended in fresh medium free of NaCl. The measurements were performed after 30 min.

NaCl Concentration	Photosynthesis	Respiration
M	$\mu mol \cdot h^{-1}\ mg^{-1}$ Chl *a*	$\mu mol \cdot h^{-1}\ mg^{-1}$ protein
0.0	440	3.0
0.1	368	3.3
0.2	289	4.2
0.3	208	4.7
0.4	173	5.8

At any steady state, an inverse relationship was evident between the photosynthetic activity and the concentration of NaCl in the medium (Table II). Nevertheless, a process of adaptation seems to have taken place, as indicated by the fact that, very soon after the exposure of cells to increased osmoticum, photosynthesis was lower (Table I) than that of cells that had attained steady state in increased osmoticum (Table II). This adaptation process may involve enhanced ion exchange and/or cellular repair (1). Another phenomenon that was typical of the response to increased osmoticum for a prolonged time was the marked increase in respiratory activity (Table II). Such an increase has already been recorded in *A. nidulans* immediately after the addition of salt, the effect being the greatest at 100 mM NaCl (5).

In agreement with Paschinger (4), we propose that the increase in the respiratory activity resulted from the increased energy requirement of the cells in a saline medium, its extent being a function of the salt concentration. This energy is, presumably, required for active excretion of the sodium ions from the cells (3) and/or for the synthesis of molecules needed for osmotic equilibration (2, 6). The suggestion that molecules that equilibrate the increased osmoticum in the growth medium accumulate during the process of adaptation to salt is supported by the marked decline in photosynthetic activity and, to a lesser extent, in endogenous respiratory activity in salt-adapted cells soon after their suspension in salt-free medium (Table III). This decline seems to indicate that the cells underwent an NaCl shock.

Our conclusion is that, in the course of adaptation to an elevation in the osmotic potential of the culture medium, two phenomena take place. First, following the initial exposure to stress, a marked decrease in photosynthetic activity occurs not associated with a change in respiratory activity. Second, after a period of adaptation, a partial reversal of the decline in photosynthetic activity becomes apparent, accompanied by a marked increase in respiratory activity.

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