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Aspects of the energetic balance of plant cells under different salt conditions ☆

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Abstract

Aspects of the intracellular energetic balance were studied in assimilating cells of *Chlorella*, grown in a medium with different concentrations of NaCl. The responses depending on salt concentration were revealed by a microcalorimetric method. The lower concentrations of NaCl (450 mM) induced an increase in the heat production by *Chlorella*. Under higher salt concentration (550 mM), a sharp increase in heat production took place. This was probably connected with the cell destruction.

In explaining the results, the protective mechanisms of *Chlorella* connected with energy redistribution under different salt conditions, are emphasized.

Keywords: Alga; *Chlorella*; Ecological stress; Energetic balance; Microcalorimetry; Salt

1. Introduction

The growth of plants under ecological stress, as well as under high and low salt conditions, requires the additional expenditure of metabolic energy [1-3]. This expenditure is necessary for maintaining electrochemical gradients, for biosynthesis of organic compounds, which play a role in protection and osmoregulation, and for

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supporting cellular structure. The higher the salt concentration the more expensive the cost of the adaptative process forming plant resistance. Adaptive changes and resistance primarily depend on bioenergetic reactions.

An objective and sensitive indicator of changes in the internal energy of living systems is their energetic balance. However, in practice, information on the change in the cell energy budget is lacking.

The aims of this work were to investigate some parameters of the energetic balance of *Chlorella* in different salt conditions and to discuss the importance of the protective mechanisms of the plants to the salt action.

2. Experimental

The single-celled alga *Chlorella* is a convenient model for investigating the energetic balance of plant cells. It was grown in Tamiya medium at 30°C [4].

The salt (final concentrations from 75 to 550 mM) was added to suspensions of the experimental variants. Cell suspensions were gassed with 0.3% CO_2 in air and illuminated with fluorescent lamps (1×10^4 lx). *Chlorella* was isolated by centrifugation at 1×10^3 rpm for 5 min; then it was resuspended in Warburg buffer with a concentration of CO_2 of 7.9×10^{-5} M.

The intensity of photosynthesis was measured as O_2 evolution by polarography using a Clark-type electrode [5]. The rate of light energy storage was determined by a differential photomicrocalorimetric method [6]. The heat production by *Chlorella* cells was measured using an LKB-2277 microcalorimeter [7].

The dynamics of the growth of *Chlorella* was measured according to the optical density of suspensions using a colorimeter [8]. The quantity of Na^+ ions in the cells was measured using flame photometry [9].

The results of gasometric and calorimetric investigations are given in energetic terms. From the photosynthesis balance equation

$$CO_2 + H_2O \xrightarrow{\text{light}} CH_2O + O_2 \qquad \Delta G_0 = +477\ \text{kJ} \tag{1}$$

where the photosynthetic coefficient is 1, the quantity of energy storage from gas exchange can be calculated.

According to Eq. (1), there is a single-valued link between O_2 evolution or CO_2 absorption and energy storage. When burning the generated product (CH_2O) 477 kJ per mole of O_2, is released. It follows that 1 ml O_2 correspond to $\approx$21 J (114 kcal: 22.4 l $\approx$ 21 kJ l^{-1} or $\approx$21 J ml^{-1}) [10].

3. Results and discussion

Fig. 1 shows that the rate of O_2 evolution by *Chlorella* cells grown on the medium with 450 mM salt decreased by more than 50% from that of the control. A new steady state was established and was supported for several hours. When this suspension was placed under optimal conditions for photosynthesis, oxygen evolu-

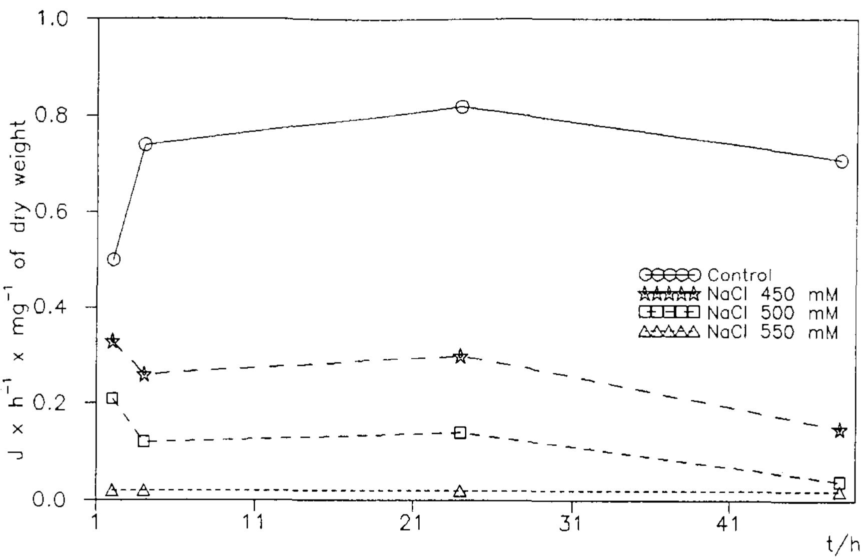

Fig. 1. Intensity of *Chlorella* photosynthesis measured by O_2 evolution under different concentrations of NaCl.

tion was rapidly restored to its control level. It can be suggested from these data that *Chlorella* became adapted to salt conditions of up to 450 mM.

At high salt content (500 mM), the plant loses its ability to adapt and passes into a stress condition. The intensity of photosynthesis is low in this condition. At 550mM salt, the culture enters a lethal state.

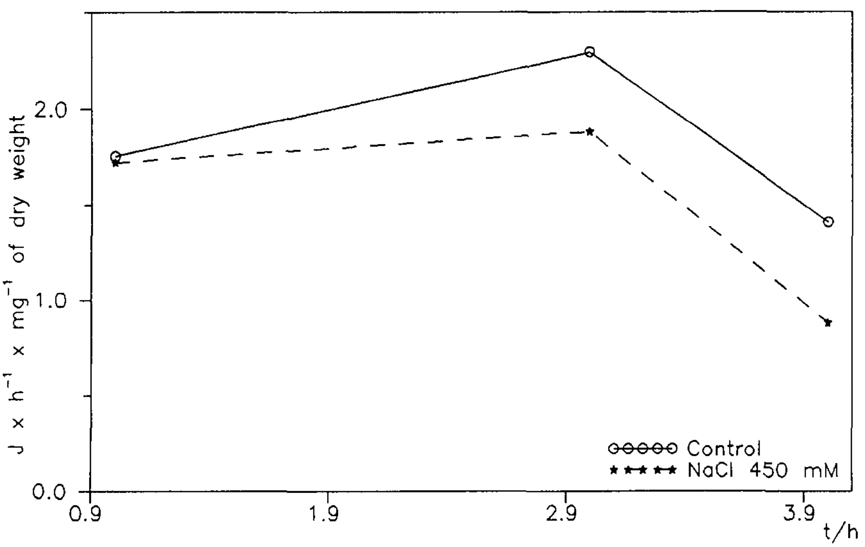

Fig. 2. The rate of light energy storage determined by photomicrocalorimetry.

The results of the direct determination of the light energy utilized by the cell measured by photomicrocalorimetry is shown in Fig. 2. In this case, the energy level of the cell is almost double that obtained by the photosynthetic gas exchange method (Fig. 1). Under salting conditions, the rate of energy storage by the algae is decreased compared with that of the control.

The highest rates of energy storage are probably connected with the work of cyclic phosphorylation, which is not accompanied by O_2 evolution. Cyclic photophosphorylation is more resistant to unfavourable environmental conditions than non-cyclic photophosphorylation [11,12]. The relative stability of endergonic processes is supposed to be protective, being an adaptive reaction of the assimilating cells allowing them to generate energy and to stabilize the energy balance.

The intensity of algal respiration grown on the medium with 450 mM salt content is twice that of the control. The increase in respiration is probably connected with its compensation role under the inhibition of photosynthesis.

As indicated in Fig. 3, 75 mM salt does not alter the rate of heat production and the results are close to those of the control. At 450 mM concentration, thermogenesis is at first higher than the control level. After some time there is a slight decrease in heat evolution. It is possible that the additional evolution of heat is connected with activation of respiration. Under these conditions, additional expenditure of metabolic energy is necessary to support cellular structure, to maintain electrochemical gradients, and in the synthesis of protective substances (for example proline) and other processes. Some inhibition of the energetic exchange at 500 mM NaCl occurs, thereby causing a decrease in thermogenesis by the *Chlorella* cells.

At high salt content in the medium (≈ 550 mM), the organism loses its ability for adaptation. The heat production by the cells increases, but the intensity of respira-

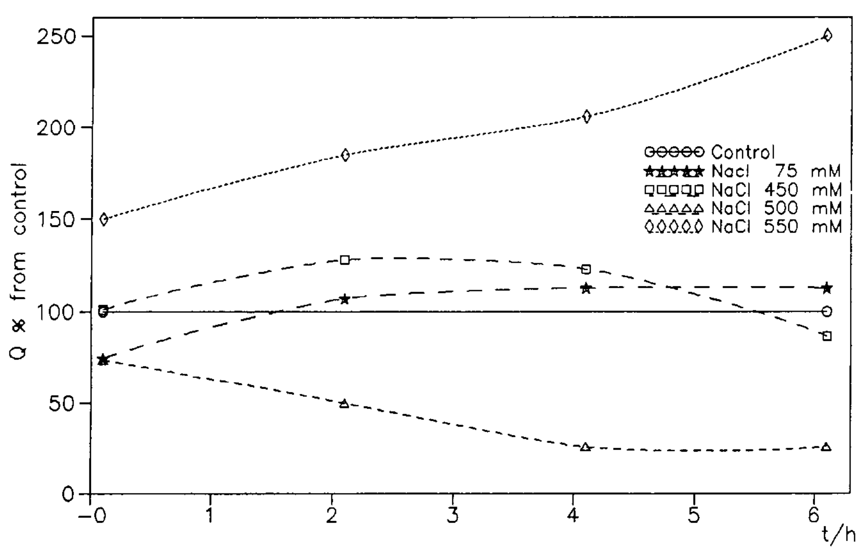

Fig. 3. The rate of heat production Q under different salt concentrations.

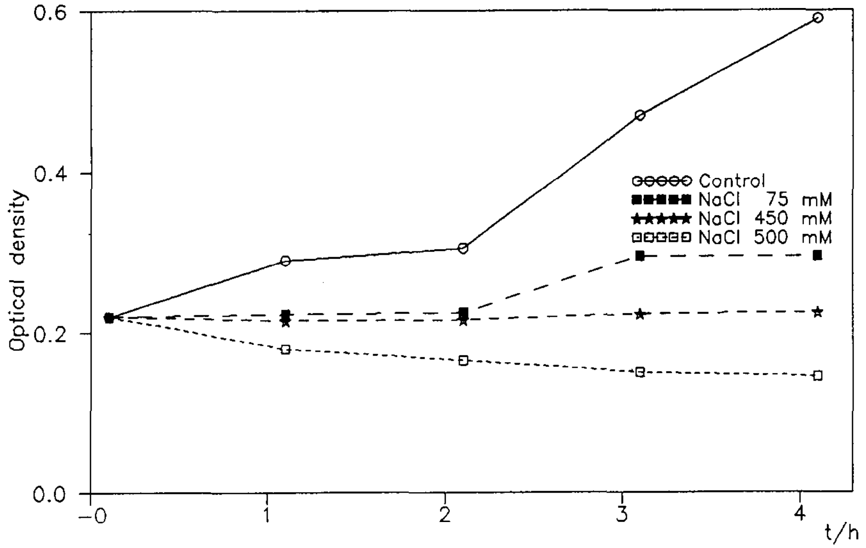

Fig. 4. The effect of salinization on the growth of *Chlorella*.

tion does not compensate for energetic losses. It is probable that the considerable heat production is due to cellular damage.

How does the cell use energy under salt conditions? Growth is the main cellular process requiring energy. Therefore, growth of the *Chlorella* suspension (Fig. 4) is an indicator of the adaptive ability of algae towards the unfavourable environmental conditions. It was shown that the cell density of the suspension decreases with increasing salt concentration so that, at 450 and 500 mM NaCl, the quantity of cells supported at low steady state is close to the initial level.

Inhibition of the growth and development processes is caused by the cessation of cell division and elongation. Probably, the cellular defence mechanisms against the saline environment do not allow the expenditure of metabolic energy for growth but only for an effective support of ion homeostasis and osmoregulation. The regulation of the cytoplasmic composition of the cell is the principal indication for the survival of the organism under these conditions.

Table 1 shows that the concentration of intracellular ions over a wide range of extracellular salt concentrations is maintained at a relatively constant level. The range of the effective regulation is 100–450 mM. At higher doses of NaCl (≈ 550 mM) there is an increase in intracellular ions.

The maintenance of a relatively stable cellular content of ions under salting depends on the work of the ionic pumps in the membranes as well as on the regulatory properties of the plasmalemma. This protective mechanism actively requires additional energy.

According to the results, the rate of O_2 evolution is considerably decreased by NaCl action. However, the energy level of the *Chlorella* cells is noticeably larger, indicating that the algae have adapted to the salt action. The growth and develop-

Table 1
Concentration of Na^+ and Cl^- ions in cytoplasm of *Chlorella* algae

NaCl concentration in the medium/mM	Ion concentration in the cell/mM	
	Na^+	Cl^-
1	8–9	10–12
100–450	18–26	18–26

ment of the cells are inhibited first. Probably, most of the energy was used for defence processes, allowing the cell to survive under these conditions.

4. Conclusions

Under "salting" conditions, the light storage of energy is supported at a rather high level.

One of the main mechanisms of salt resistance in microalgae is energy redistribution. The energy expenditure for growth and development of the culture decreases, whereas that for maintaining cellular structure and for supporting ion homeostasis, osmoregulation and its attendant processes increases. This energy redistribution in the plant cell can be regarded as the protective and adaptive reactions of the organism, which allows it to survive in salting conditions.

Investigation of the processes of energy storage and energy expenditure contributes to an understanding of the mechanisms of the formation of the adaptation potential and plant productivity.

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