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Salt stress impact on the molecular structure and function of the photosynthetic apparatus—The protective role of polyamines

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Abstract

In the present study the green alga *Scenedesmus obliquus* was used to assess the effects of high salinity (high NaCl-concentration) on the structure and function of the photosynthetic apparatus and the possibility for alleviation by exogenous putrescine (Put). Chlorophyll fluorescence data revealed the range of the changes induced in the photosynthetic apparatus by different NaCl concentrations, which altogether pointed towards an increased excitation pressure. At the same time, changes in the levels of endogenous polyamine concentrations, both in cell and in isolated thylakoid preparations were also evidenced. Certain polyamine changes (Put reduction) were correlated with changes in the structure and function of the photosynthetic apparatus, such as the increase in the functional size of the antenna and the reduction in the density of active photosystem II reaction centers. Thus, exogenously added Put was used to compensate for this stress condition and to adjust the above mentioned changes, so that to confer some kind of tolerance to the photosynthetic apparatus against enhanced NaCl-salinity and permit cell growth even in NaCl concentrations that under natural conditions would be toxic.

Keywords: Photosynthetic apparatus; Polyamines; Salinity; Environmental stress; Chlorophyll fluorescence

1. Introduction

Salinity is a major abiotic stress in plants worldwide. Salt stress causes an initial water-deficit, due to the relatively high solute concentrations in the soil, and also ion-specific stresses resulting from changes in K^+/Na^+ ratios. Thus, it leads to increased Na^+ and Cl^- concentrations that are detrimental to plants [1,2]. Salt-stressed plants exhibit a decrease in their photosynthetic efficiency, but it is not known how this actually occurs [3]. There is increasing evidence that enhanced salinity changes photosynthetic parameters, osmotic and leaf water potential, transpiration rate, leaf temperature, and relative leaf water content [4]. Salt also affects photosynthetic components such as enzymes, chlorophylls, and carotenoids. Changes in these parameters depend on the severity and duration of the stress [5] and also on plant species [6]. Reduced photosynthesis with increasing salinity has been attributed to: (a) stomatal closure, which leads to a reduction in intracellular CO_2 partial pressure; (b) non-stomatal factors [7,8], as the reduction in protein concentration [9]; (c) decline in photosynthetic pigments [4,10]; and (d) changes in ionic concentrations [11].

A significant inhibition of photosynthesis by high salinity seems to be associated with the photosystem II (PSII) complex. Salinity stress decreases the PSII activity [12] and inhibits the quantum yield of PSII electron transport [13]. The inhibitory target site of high salt concentration at PSII seems to vary with species [14,15]. Nevertheless, Misra et al. [16] have reported that NaCl salinity affects PSII photochemical efficiency, primary charge separation in PSII and pigment–protein complexes of thylakoid membranes. Allakhverdiev et al. [17] analysed Chl fluorescence in salt-stressed cyanobacterium

Abbreviations: Chl, chlorophyll; LHCII, light harvesting complex of PSII; PCV, packed cell volume; PS, photosystem; Put, putrescine; Spd, spermidine; Spm, spermine

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S*ynechococcus* sp., and suggested that the photochemical reaction center complex, including Q_A (the primary electron acceptor of plastoquinone), pheophytin, and the photochemical reaction center P680, was undamaged in NaCl-treated cells. Therefore, it is likely that the transport of electrons from water to P680 is blocked in these cells. NaCl interfered with the PSII-mediated transport of electrons from water to DCIP, but not from DCP to DCIP. Thus, it is likely that the oxygen-evolving machinery in PSII is damaged by the ionic effects [18].

On the other hand there have been a couple of publications reporting the importance of high endogenous polyamines and especially of putrescine to the stress tolerance of plants against high salinity [19,20]. However, there is no publication investigating the role of polyamines to the stress tolerance of the photosynthetic apparatus. Thus, the object of the present study is to investigate the effects of salinity stress on the molecular structure, function and bioenergetics of the photosynthetic apparatus and the possibility that polyamines are able to regulate this salinity effect.

2. Materials and methods

2.1. Cultures

Cultures of the unicellular freshwater green alga *Scenedesmus obliquus*, wild type strain D3 [21], were grown autotrophically in liquid culture medium [22] in a temperature-controlled water bath (30 °C) in front of a set of white fluorescent lamps (OSRAM L-40W, Munich, Germany). Cultures were grown at high light (HL: 270 μmol m^{-2} s^{-1}) intensities. The cultures were continuously percolated with air for CO_2 supply and also to avoid sedimentation. The initial cell concentration for each culture was 0.3 μl PCV ml^{-1}. Eight cultures were used for the NaCl treatment with different NaCl concentrations [0‰ (control), 2‰, 5‰, 7‰, 10‰, 15‰, 20‰, and 25‰ NaCl] in high light intensity of 270 μmol m^{-2} s^{-1}. For the Put-supplemented cultures, a stock solution (100 mM) of the polyamine putrescine (Tetramethylenediamine, Sigma) in water was first prepared. Aliquots of this solution were added to the cultures to achieve a final concentration of 1 mM.

2.2. Isolation of the thylakoid membranes

For the preparation of thylakoid membranes the algal cultures were centrifuged for 5 min at 1500×*g* and the pellets resuspended in 75 mM phosphate buffer (pH 7.4). The suspension was mixed with glass beads (Ø 0.2 mm) and broken 4 times for 1 min in a cell mill (Biospec, OK, USA). The homogenate was filtered through a sinter glass filter funnel to separate the glass beads, and centrifuged for 2 min at 500×*g* to remove unbroken cells and debris. The supernatant was additionally centrifuged for 60 min at 8000×*g*. The pellet

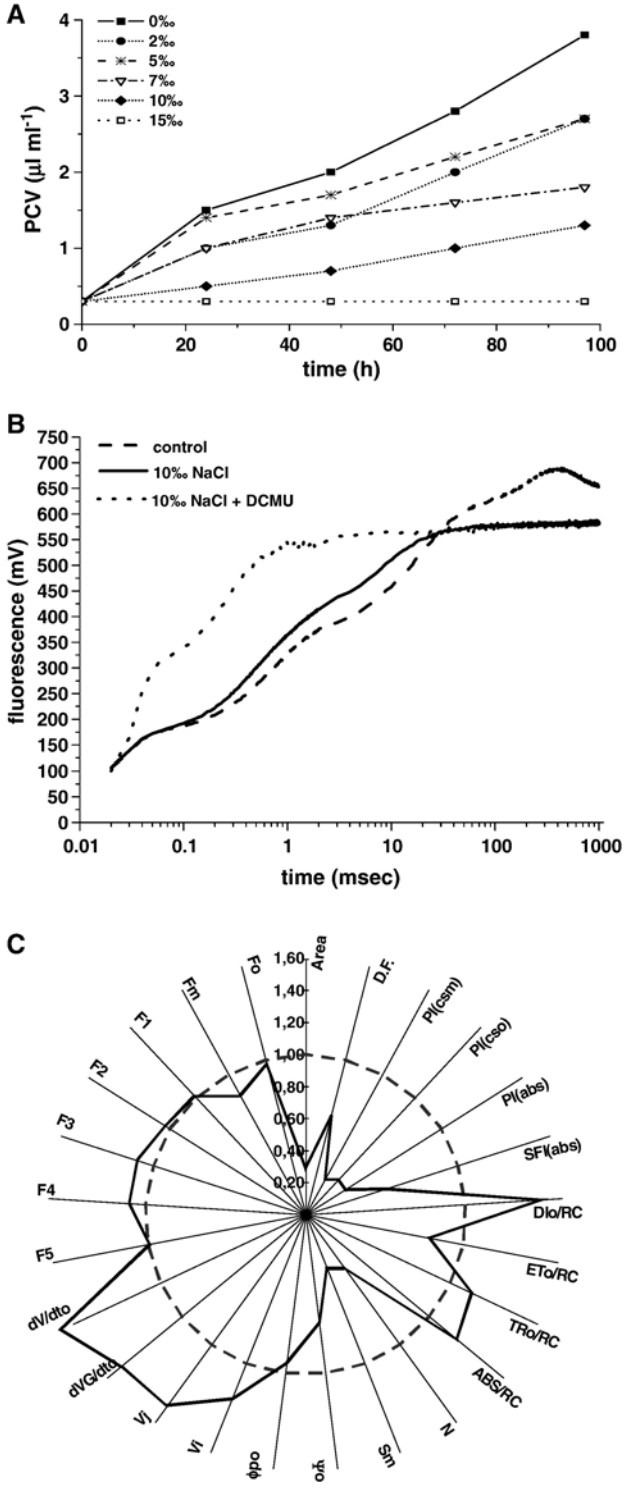

Fig. 1. (A) Culture growth measured as packed cell volume (PCV) in *Scenedesmus obliquus* autotrophic cultures. Control culture received no additional treatment, whereas the other five cultures were supplemented with NaCl upon inoculation, in different concentrations (2‰, 5‰, 7‰, 10‰, 15‰). (B) Fluorescence curves recorded from the control and the 10‰ NaCl-supplemented cultures, 96 h after onset of the experiment. The Fm value of the NaCl-treated sample was also confirmed by DCMU inhibition. (C) Radar plot with a series of parameters derived from JIP-test analyses of the fluorescence data. The plot depicts the differences provoked in the structure and function of the photosynthetic apparatus under enhanced salinity (10‰ NaCl culture; black line) in comparison to the physiological condition (control culture; grey circle). Area: the total complementary area (from time 0 to t_{Fm}) over the fluorescence induction curve is a measure of the number of quanta not emitted as fluorescence as a consequence of the photochemistry during the induction phase; D.F.: Driving Force of photosynthesis; PI: the performance indexes; SFI: the structure function index; DIo/RC: dissipation energy per active reaction center; ETo/RC: electron transport per reaction center; TRo/RC: the trapping efficiency per reaction center; ABS/RC: the effective antenna size; *N*: expresses how many times Q_A has been reduced in the time span from time 0 to t_{Fm}; Sm: a measure of the energy needed to close all reaction centers; ψ_o: the primary photochemistry; φpo: the photosynthetic efficiency; V_i: variable fluorescence at 30 ms; V_j: variable fluorescence at 2 ms; dVG/dto: expresses the excitation energy transfer between the RCs; dV/dto: expresses the rate of the RCs' closure; F1: $F_{50\ \mu s}$; F2: $F_{100\ \mu s}$; F3: $F_{300\ \mu s}$; F4: $F_{2\ ms}$; F5: $F_{30\ ms}$; Fm: Maximal fluorescence (measured when the reaction centers are closed, at P step); Fo: Initial fluorescence (measured when the reaction centers are open, at O step of the fluorescence induction curve).