INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-4 Oct-Dec-2016 Coden: IJPAJX-CAS-USA, Copyrights@2015ISSN-2231-4490
Received: 5th Aug-2016 *Revised: 20th Sept -2016* *Accepted: 22nd Sept-2016*
DOI: 10.21276/Ijpaes *http://dx.doi.org/10.21276/ijpaes* *Research article*

CHARACTERIZATION OF LOW SALT STRESS INDUCED ALTERATIONS IN THE PHOTOSYNTHETIC ELECTRON TRANSPORT IN *SPIRULINA PLATENSIS*

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ABSTRACT: In this investigation an attempt has been made to characterize the alterations in photosynthetic parameters by depriving salt (NaCl) from the growth medium of the cyanobacterium *Spirulina platensis* and also by re-addition of salt after different intervals (0-42 min). Salt deprivation primarily affected the photosystem (PS) II catalysed electron transport. Most probably due to the alterations in water oxidation complex could be responsible for the altered PS II photochemistry. Experiments related to the re-addition of salt also suggested the recovery of the loss in PS II activity after 42 min of incubation. Fluorescence kinetic measurements made by pulse amplitude modulator (PAM) kinetic fluorimeter also supported the above observation in the above cyanobacterium.
Key words: Chlorophyll *a* fluorescence, Cyanobacterium, Electron transport, Photosystem, Spectral properties.

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INTRODUCTION

The significance of sodium salts for cyanobacteria has been demonstrated in several cases including growth nitrogen fixation, the uptake of nutrients such as nitrate and phosphate [1] and energy transduction. In cyanobacteria, several studies revealed that, sodium salt deficiency was attributed to the inhibition of bicarbonate transport [2]. In some cyanobacteria sodium salt has direct effects on photosynthesis. A decrease in the cellular content of both Chl and PBPs was observed in the sodium salt deficient medium. The loss in photosynthetic net oxygen evolution was observed in *Synechocystis* sp. PCC 6714 [3], in alkaliphilic cyanobacterium *Spirulina platensis* [4] and in alkalitolarent cyanobaterium *Synechococcus leopoliensis* up-on the sodium salt deprivation from growth medium. The effect of loss in PS II activity due to sodium salt deprivation is reversible by the addition of Ca^{2+} and Na^{+} in *Synechocystis* [3]. It has also been demonstrated that Na-ions play important role in cyclic PS I electron transport under stress conditions [5]. A group of cyanobacteria living at alkaline pH was characterized by Langworthy, (1978) [6]. These cyanobacteria, living at alkaline conditions, require sodium salt to maintain acidic intracellular pH relative to the external alkaline pH. It has been demonstrated that, alkaliphliles could maintain the intracellular pH by exchange of cytoplasmic Na^{+} for external H^{+}, which is maintained by Na^{+}/H^{+} antiporter activity and thereby prevent the loss of all physiological and metabolic activities. *Spirulina platensis* is an obligate alkaliphilic cyanobacterium and it requires 150-250 mM of sodium salts for optimal growth. In the present study, an attempt has been made to study the requirement of low sodium salts for photosynthesis and pH homeostasis in this cyanobacterium.

MATERIALS AND METHODS

Crude thylakoid membranes was prepared according to Hagio *et al.* (2000) [7] and Nishiyama *et al.* (1993) [8] with some modifications. Whole chain electron transport activity was measured in whole cells and thylakoids by using methylviologen (MV) as terminal electron acceptor ($H_2O \rightarrow MV$). Whole cells and thylakoids equivalent to 12 µg and 10 µg of Chl *a* were used respectively. Photosynthetic net oxygen evolution was measured in whole cells. PS II activity was measured in whole cells and thylakoids by using freshly prepared 0.5 mM pBQ (para-benzoquinone) as an electron acceptor of PS II ($H_2O \rightarrow$ pBQ) [9].

PS I activity was measured by using DCPIP (reduced by Asc), serve as artificial electron donor to PS I, and MV was used as an exogenous terminal electron acceptor (DCPIPH2→ MV). DCMU, 3 (3,4-dichlorophenyl)-1, 1-demethyl urea) was used to block the electron flow from PS II to PS I. 0.1 mM DCPIP, 5 mM ascorbate, 10 µM DCMU, 50 µM MV, 5 mM sodium azide were used for the PS I electron transport assay [9]. Induction of Chl *a* fluorescence was measured with pulse-modulated fluorimeter (PAM101, Heinz Walz GmbH, Effeltrich, Germany), which was developed by Dr. U. Schreiber. The fluorescence minimum (Fo) and fluorescence maximum (Fm) were measured according to Campbell *et al*. (1998) [10]. The Fo was measured with a weak modulated light intensity (1 mWm^{-2}). The Fm level of fluorescence was recorded during 1 s saturating white light pulses obtained from a halogen lamp (KL-1500 Electronic, Schott Glasswerke, Wiesbaden, Germany). The efficiency of PS II was calculated from Fm and Fo according to Campbell *et al.* (1998) [10].

RESULTS AND DISCUSSION

The cyanobacterium *Spirulina platensis* living at alkaline pH (9.0-11.5) requires 150-250 mM Na-ions for optimal growth [9]. Krulwich and Guffanti (1989) [11] demonstrated that *Spirulina platensis* is an obligate alkaliphilic cyanobacterium and it could not grow at neutral pH and in the absence of sodium salts.

In the present study, upon the deprivation of sodium salt at alkaline pH the inhibition in net oxygen evolution and whole chain electron transport activity was observed in *Spirulina platensis*. The loss in whole chain electron transport activity could be due to the inhibition at water oxidation complex or PS II reaction centre or PS I. To elucidate the target in electron transport, the partial photochemical reactions were measured by using artificial electron donors and acceptors. The restoration of the whole chain electron transport activity by DPC, which is an electron donor to PS II, suggested that water oxidation complex is the target site for sodium salt deprivation. However, PS I and intersystem mediated electron transport activities were not affected by salt deprivation as shown in the results (Table 1). Restoration of PS II activity by DPC, an artificial electron donor to PS II in salt treated cyanobacterial thylakoids suggests that water splitting complex is the site of action of salt stress in *Synechococcus* [12]. Table 2 shows the time dependent recovery of O_2 evolution in PS II upon the addition of 200mM NaCl. Maximum recovery was noticed after 42 min of salt re-addition suggesting the repair of PS II at water oxidation complex.

Further-more, PS II efficiency measured as Chl *a* induction kinetics also shown the loss in $Fv/Fm_{(dark)}$ upon the shift of cells to the sodium deficient medium. This could be due to the inhibition of PS II electron transport [13] (Fig:1). After the re-addition of sodium, 80% of the restoration was observed within 50min (Fig:2). Zhao and Brand (1989) [3] have showed that the restoration of photosynthesis is dependent on the time factor of the sodium re-addition i.e., longer exposure to Na^+ deprivation (stress) causes more severe damage to the photosynthetic apparatus.

Table 1: Effect of salt deprivation on the electron transport activities measured in whole cells.

Electron transport assay	Electron transport activity (µ moles of O_2 consumed or evolved mg Chl^{-1} h^{-1})		
	Control	Treated	Percentage loss
H2O→MV	128	20	84
DPC→MV	235	115	15
DQH2 →MV	128	110	14
DCPIP→MV	210	200	5

Table 2: Time dependent effect of salt re-addition (200 mM NaCl) on photosynthetic net oxygen evolution activity of *Spirulina* cells.

Duration after re-addition of salt(min)	PS II catalysed electron transport (µ moles)	Recovery Percentage
0	23	0
6	110	50
24	176	80
42	220	100

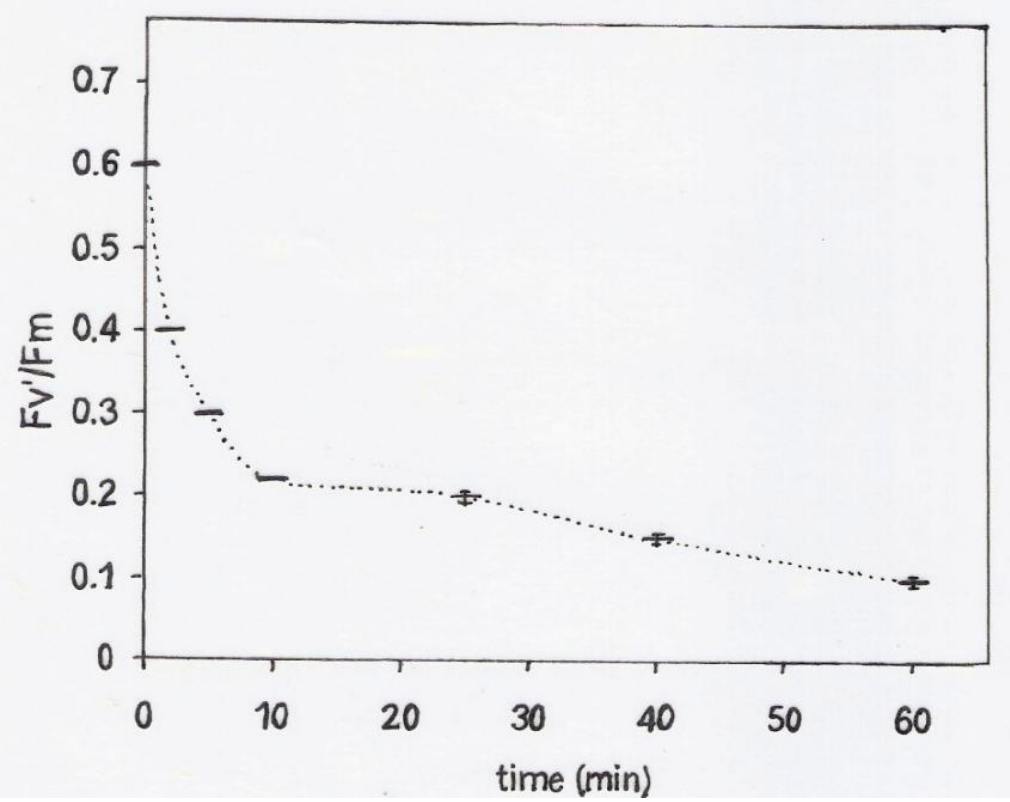

Fig:1 Time dependent effect of salt deprivation (0 mM Nacl) on maximal efficiency ($Fv/Fm_{(dark)}$) of PS II. ($Fv/Fm_{(dark)}$) was measured in whole cells by PAM fluorimetry. Other details were given in materials and methods. The values are average of 3 separate experiments. The SD is not more than 9%.

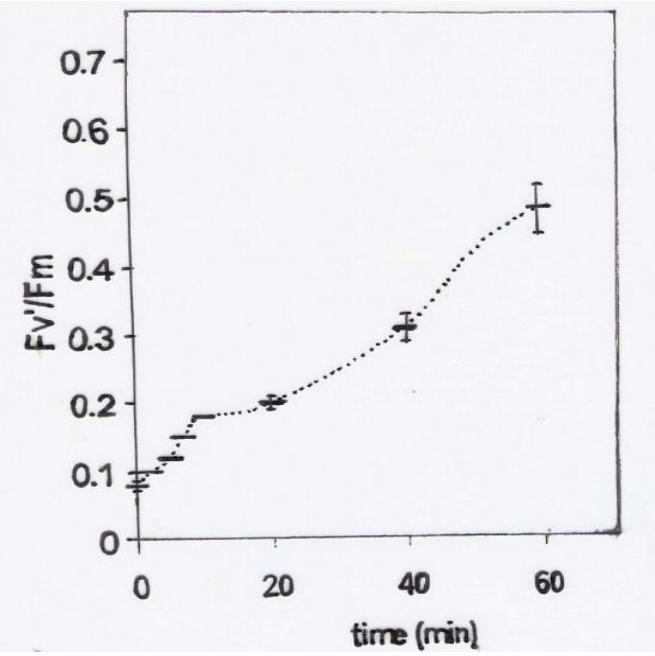

Fig: 2 Time dependent effect of salt re-addition (200 mM Nacl) on maximal efficiency ($Fv/Fm_{(dark)}$) of PS II. ($Fv/Fm_{(dark)}$) was measured in whole cells by PAM fluorimetry. Other details were given in materials and methods. The values are average of 3 separate experiments. The SD is not more than 9%.

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INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences