Biochimica et Biophysica Acta 1857 (2016) 715–722

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Towards clarifying what distinguishes cyanobacteria able to resurrect after desiccation from those that cannot: The photosynthetic aspect

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ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form 26 January 2016
Accepted 13 February 2016
Available online 16 February 2016

Keywords:
Dehydration
Drought stress
Fluorescence
Photosynthetic activity
Photosystem II
Q_A-reoxidation

ABSTRACT

Organisms inhabiting biological soil crusts (BSCs) are able to cope with extreme environmental conditions including daily hydration/dehydration cycles, high irradiance and extreme temperatures. The photosynthetic machinery, potentially the main source of damaging reactive oxygen species during cessation of CO_2 fixation in desiccating cells, must be protected to avoid sustained photodamage. We compared certain photosynthetic parameters and the response to excess light of BCS-inhabiting, desiccation-tolerant cyanobacteria *Leptolyngbya ohadii* and *Nostoc reinholdii* with those observed in the "model" organisms *Nostoc* sp. PCC 7120, able to resurrect after mild desiccation, and *Synechococcus elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 that are unable to recover from dehydration. Desiccation-tolerant strains exhibited a transient decline in the photosynthetic rate at light intensities corresponding to the inflection point in the PI curve relating the O_2 evolution rate to light intensity. They also exhibited a faster and larger loss of variable fluorescence and profoundly faster Q_A^- re-oxidation rates after exposure to high illumination. Finally, a smaller difference was found in the temperature of maximal thermoluminescence signal in the absence or presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) than observed in "model" cyanobacteria. These parameters indicate specific functional differences of photosystem II (PSII) between desiccation tolerant and sensitive cyanobacteria. We propose that exposure to excess irradiation activates a non-radiative electron recombination route inside PSII that minimizes formation of damaging singlet oxygen in the desiccation-tolerant cyanobacteria and thereby reduces photodamage.

1. Introduction

Biological soil crusts (BSC) play an important role in stabilizing sandy areas and influence the biotic composition of arid and semiarid ecosystems [1,2]. The crusts are formed by the adhesion of the sand particles to extracellular polysaccharides (EPS) secreted mostly by filamentous cyanobacteria. These organisms are the pioneers and main primary producers in BSCs where they must cope with extreme temperatures, high irradiance and frequent hydration/dehydration cycles [3–8]. Many filamentous cyanobacteria are able to form special dormant cells, akinetes, which can withstand long desiccation periods and germinate after rewetting [9,10]. However, unlike BSC-inhabiting organisms, their vegetative cells are unable to survive such daily hydration/desiccation cycles. Most cyanobacteria used as model organisms, for which genetic tools have been developed, are unable to recover after frequent dehydration. In contrast, others such as *Nostoc* sp. PCC 7120 (hereafter *Nostoc* 7120), often used as a model organism in N_2 fixation and differentiation (heterocyst development) studies [11–13], can withstand mild desiccation [14–18] but not rapid and repeated hydration/desiccations experienced by specialized cyanobacteria in desert crusts.

The mechanisms involved in the ability to cope with frequent hydration/desiccation cycles are largely unknown [19]. The pioneering studies of Potts and colleagues [3,20] on the acclimation of *Nostoc commune* to desiccation suggested that stabilization of existing proteins during dehydration must be involved to enable resurrection under favorable conditions. It is also likely that the ability to reversibly activate metabolism and grow when water is available, and to shutdown metabolic activity during dehydration, plays an important part in this acclimation.

Abbreviations: BSC, biological soil crusts; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PSII, photosystem II.

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http://dx.doi.org/10.1016/j.bbabio.2016.02.007
0005-2728/

Analysis of the transcriptome using DNA microarray showed that *Nostoc* 7120 activates a certain set of genes after dehydration including those for the synthesis of trehalose, a compatible solute efficiently protecting desiccated organisms, potassium transporters and genes possibly involved in EPS synthesis [17].

The photosynthetic machinery is potentially a major source of damaging reactive oxygen species (ROS), particularly when exposed to excess illumination higher than required to saturate CO_2 fixation [21–26]. The production of ROS may rise with increasing light intensity during desiccation in the field [27]. ROS-mediated damage could be responsible for declining photochemical activity because of impaired ability to repair photodamage in the photosynthetic reaction centers in the drying cell. Hence, the mechanisms whereby desiccation-tolerant cyanobacteria regulates the photosynthetic system upon hydration may be of importance to reduce the ROS load during desiccation [28,29] and protect themselves against photodamage under excess illumination [23,30–32], particularly during dehydration [19]. An earlier study [27] showed that activation of photosynthesis during hydration of a BSC did not require *de novo* protein synthesis. Energy transfer to the reaction centers and electron transfer activities were observed within a few minutes following hydration. Our earlier studies on *Microcoleus* sp. isolated from the BSC showed remarkable resistance to photoinhibition, even in the absence of protein synthesis. These studies brought evidence indicating the activation, by light, of a non-radiative cyclic electron transfer route within PSII that serves as a protection mechanism from excess illumination [30,31].

Our long-term aim is to gain a better understanding of what distinguishes cyanobacteria able to resurrect after desiccation from those that cannot. In view of the earlier results, in the present study we compared the photosynthetic responses of two BCS-inhabiting, desiccation-tolerant cyanobacteria with those observed in three "model" aquatic organisms that were unable to resurrect after BSC-like dehydration. Our integrated analysis of photosynthesis parameters represents the first step towards recognition of functions allowing specialized cyanobacteria to thrive in BSCs.

2. Experimental procedures

2.1. Organisms and growth conditions

BSC samples were collected from Nizzana (N30°56′25″; E34°22′55″) and Wadi Paran (30°19′35″N 34°57′16″E(, Negev desert, Israel. Several filamentous cyanobacteria were isolated including *Leptolyngbya ohadii* and a *Nostoc reinholdii* used in the present study. *Synechocystis* sp. strain PCC 6803, *Synechococcus elongatus* PCC 7942 and *Nostoc* sp. PCC 7120 are routinely maintained in our laboratory. The cyanobacteria were cultivated in BG11 medium [33] at 30 °C under LED light (3000 °K, 100 μmol photons m^{-2} s^{-}) with continuous air bubbling. When necessary, the aggregated cells suspension of *Leptolyngbya* was homogenized in BG11 medium.

2.2. Excess illumination treatments

These were carried out with cell suspension placed in a temperature-controlled home-made chamber surrounded with computer-controlled LEDs (3000 °K) at 30 °C with the desired light intensity and with continuous air bubbling. Samples were subsequently taken to an oxygen electrode and fluorometer.

2.3. O_2 evolution and chlorophyll fluorescence of culture

Oxygen evolution was measured using a Clark type O_2 electrode (PS2108, Passport dissolved O_2 sensor Roseville, CA, USA) and PAM fluorometer (Walz PAM 2500, Effertlich, Germany). Cyanobacteria cells corresponding to 10 μg ml^{-1} chlorophyll suspended in fresh medium were incubated in a light and temperature-controlled glass holder (optical path 1.9 cm, LED light 3000 °K, 30 °C) for at least 1 h in low light (50 μmol photons m^{-2} s^{-1}) before the experiment. Then, illumination of 2000 μmol photons m^{-2} s^{-1} was applied for 1 h with or without 5 mM HCO_3^- (continuous air bubbling used in both cases) and 1 ml samples were withdrawn every 15 min for O_2 exchange and fluorescence measurements. Fluorescence emission measurements were performed using fibre-optics for the PAM fluorometer after 2 min of dark adaptation. For determination of the response of O_2 evolution to light intensity, the cells were illuminated for 3 min under the desired photon flux density using the LED light.

2.4. Thermoluminescence (TL) emission

TL was measured as described [31,34]. Briefly, samples (4–5 μg chlorophyll, 0.4 ml) were dark-adapted at 25 °C, rapidly frozen to -22 °C and excited by 2 single turnover light flashes (3 μs, xenon arc discharge). The samples were then heated at a rate of 0.6 °C s^{-1} to 50 °C while counting photon emissions (B band). For detection of the Q band, 10 μM, the herbicide DCMU (Sigma, Aldrich, Germany), which binds to the Q_B site, was added before dark-adaptation with a concentration that completely inhibits oxygen evolution in these organisms.

2.5. Fluorescence and kinetics of Q_A^- re-oxidation

These were measured using a PAM fluorometer (Walz PAM 2500, Effertlich, Germany) and a Joliot type spectrophotometer JTS-10 (Bio-Logic, Claix, France), respectively. Saturating flashes (30 μsec) in the JTS-10 were obtained with a green LED flash and blue LEDs for measuring light on cells that were dark-adapted.

3. Results

3.1. Rationale

To reveal adaptive differences in the function of the photosynthetic apparatus, we compared the response of the photosynthetic machinery to high illumination in five different cyanobacteria distinguished by their ability to recover growth after desiccation. Two filamentous cyanobacteria were isolated from a BSC in the Israeli desert, a *Leptolyngbya* sp. that we named *L. ohadii* in honor of Professor Itzhak Ohad (hereafter *Leptolyngbya*), and a *Nostoc* sp. that we named *N. reinholdii* in honor of Professor Leonora Reinhold (hereafter *Nostoc*). Two unicellular cyanobacteria often used as a model system, *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis*) and *S. elongatus* PCC 7942 (hereafter *Synechococcus*), are both unable to recover after dehydration. It was earlier shown that *Nostoc* 7120 is able to resurrect after desiccation under mild laboratory conditions, 28 °C, low light intensity and 70% humidity [14]. Using our environmental chamber, which enables accurate and reproducible simulation of the conditions in the BSC [8] clearly indicated that *Nostoc* 7120 is unable to revive after exposure to the desert-kind dehydration. Neither could a mutant thereof that produces much more EPS than does the wild type [35], also examined using the environmental chamber (not shown).

3.2. Photosynthetic parameters

Below we provide examples of the data obtained from four sets of experiments where we examined certain aspects of the photosynthetic performance of the five organisms investigated here (Figs. 1–3) and a summing up (Fig. 4) comparing the performance of these organisms.

3.2.1. Light dependence of the photosynthetic rate in the various strains

To characterize the dynamic range of the photosynthetic response to illumination, we performed "classical" measurements of the rate of CO_2-dependent O_2 evolution as affected by the incident light intensity.