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Probing the effects of high-light stress on pigment and lipid metabolism in nitrogen-starving microalgae by measuring chlorophyll fluorescence transients: Studies with a Δ5 desaturase mutant of *Parietochloris incisa* (Chlorophyta, Trebouxiophyceae)

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ABSTRACT

We investigated effects of irradiance on the relationships between chlorophyll fluorescence transients (OJIP), carotenoid-to-chlorophyll ratio, and fatty acids in a nitrogen-deprived *Parietochloris incisa* (Chlorophyta, Trebouxiophyceae) Δ5 desaturase mutant accumulating valuable LC-PUFA dihomo-γ-linolenic acid (DGLA). High light (270 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR) and nitrogen starvation brought about a decrease in maximum quantum yield of photosystem II (Φ_{P_0}) and electron transport (Φ_{E_0}) but enhanced the quantum yield of thermal dissipation (Φ_{D_0}) and induced non-photochemical quenching (NPQ) in an irradiance-dependent manner. Under high irradiance a decline in the rate of total fatty acid accumulation and DGLA percentage in comparison with the cultures grown under 130 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR was recorded. Increasing irradiance from 130 to 270 $\mu E \cdot m^{-2} \cdot s^{-1}$ enhanced total fatty acid accumulation only within the first week of nitrogen starvation and negatively affected DGLA production. Regardless of irradiance, Φ_{P_0}, Φ_{E_0}, and Φ_{D_0} exhibited tight ($r^2 = 0.8–0.9$) relationships with the stress-induced changes of total fatty acid and DGLA content and the carotenoid-to-chlorophyll ratio. The applicability and limitations of OJIP and its derived parameters for on-line monitoring of physiological condition and accumulation of value-added products in microalgal cultures grown in photobioreactors are discussed.

1. Introduction

In various groups of microalgae environmental stresses such as high PAR irradiance and/or nitrogen deficiency bring about profound changes in photosynthetic apparatus (PSA) structure and function as well as in lipid and pigment metabolism [1]. In many cases a build-up of storage triacylglycerols (TAG) coordinated with a decline in chlorophylls (Chl) and accumulation of secondary carotenoids (Car) takes place [2,3]. These phenomena have important biotechnological implications since Car and fatty acids (FA) accumulated by certain microalgae under the stress condition are high-value nutraceuticals and antioxidants; neutral lipids are considered as a promising feedstock for biodiesel [4,5].

The high-light acclimation of microalgal cells involves engagement of photoprotective mechanisms decreasing the absorption of light by the PSA and enhancing thermal dissipation of the absorbed light energy within PSA [1]. In mass culture of microalgae, understanding the relationships of the adaptive re-arrangements of the PSA with lipid metabolism is essential for the estimation of stress intensity of oleaginous species. It is also important for timely adjustment of cultivation conditions enabling the organism to cope with the stress, to achieve high yields of target products, and to make the whole process more sustainable.

Analysis of chlorophyll fluorescence (CF) is a powerful tool for revealing physiological state of photoautotrophic organisms [6,7]. Light-induced thermal energy dissipation in PS II antenna can be examined by measuring non-photochemical CF quenching with a Pulse Amplitude Modulated (PAM) fluorometer. Even more information can be obtained by recording high-resolution light-induced kinetics of Chl fluorescence (OJIP transients) [8]. OJIP transient reflects changes in electron transport in PS II over a wide range of time from microseconds to seconds that allows to evaluate such important characteristics of PS II as energy trapping, electron transport, and ΔpH-dependent dissipation of excitation energy into heat in the antenna complex [6]. It provides a valuable insight into the transformation of the absorbed light energy within PSA, efficiency of its photochemical utilization and engagement of photoprotective

Abbreviations: Car, carotenoid(s); Chl, chlorophyll(s); CF, chlorophyll fluorescence; DGLA, dihomo-γ-linolenic acid; DW, dry weight; OB, oil bodies; PAR, photosynthetically active radiation; PSA, photosynthetic apparatus; TAG, triacylglycerols; (T)FA, (total) fatty acids; PUFA, polyunsaturated fatty acids; WT, wild type.

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mechanisms [9]. Other CF-related parameters such as NPQ related with non-photochemical dissipation of the absorbed light and F_V/F_M, the maximum quantum yield of photosystem (PS) II photochemistry [10], are commonly utilized in monitoring of the growth [11] and physiological condition of microalgae as well.

A major practical advantage of using CF is that the measurements are non-destructive, rapid, and easy to perform. These characteristics make CF analysis a particularly attractive method for on-line monitoring of the physiological condition of planktonic [8,12] and cultivated microalgae [13]. There are many reports on application of CF-based parameters for *in situ* monitoring of productivity of and adaptation to light in mass cultured microalgae ([13] and references therein). However it is difficult to link the CF-derived parameters with remote to primary photochemistry characteristics of a culture such as cell dry weight or FA content and composition since they are affected by many environmental and intrinsic factors which are not directly related with PSA.

In spite of these difficulties, CF-based approach for on-line monitoring of key parameters of an algal culture would be welcomed by microalgal biotechnologists developing processes for the production of high value bioproducts and biofuels as well as biomitigation of wastes. An emphasis should be put to the assessment of photosynthetic capacity of cultivated algae *in situ* under nitrogen starvation conditions since it increases the vulnerability of the culture to photooxidative damage under higher irradiances.

Towards this end, we made an attempt to map selected CF-based parameters to the performance (biomass, total FA, DGLA, and Car accumulation) of a green oleaginous microalga cultivated under conditions of different stress intensity. A Δ5 desaturase mutant of *Parietochloris incisa* (P127) obtained in the Laboratory of Microalgal Biotechnology (Ben-Gurion University, Israel), served as an object in the present work. Due to the nonsense mutation in the Δ5 desaturase gene the strain is almost incapable of desaturation of dihomo-γ-linolenic acid (DGLA, 20:3ω−6) to arachidonic acid (AA, 20:4ω−6) [14]. Under nitrogen starvation conditions the mutant accumulates high contents of DGLA that is mainly incorporated in triacylglycerols (TAG) up to 39% of total fatty acids (TFA) and 14% of dry weight (DW) [3]. In fungi and algae and invertebrates DGLA normally occurs only as an intermediate in AA biosynthesis and does not accumulate in any appreciable amounts. DGLA has a pharmacological significance related with its anti-inflammatory activity such as for treating atopic eczema, psoriasis, asthma and arthritis [15]. Recent studies suggest that DGLA possesses antiproliferative properties and is unique among the ω−6 polyunsaturated FA family members in its potential to suppress tumor growth and metastasis [16]. Therefore, the mutant could serve as a potential source of the nutraceutically important LC-PUFA.

In this context it was important to study the relationships between FA and pigment composition, FA accumulation and parameters of OJIP curves characterizing photosynthetic performance of the mutant strain under conditions favoring DGLA accumulation (nitrogen deficiency and high irradiance). Previously we have described in detail the major patterns in biomass and fatty acid accumulation by this strain grown on N-replete and N-depleted media under different irradiance levels and revealed tight correlations between FA accumulation, changes in pigment composition, and optical absorbance of the algal cell suspension [3]. In this paper we focus on OJIP curves in the stressed mutant cells grown under nitrogen starvation and different PAR irradiances. To the best of our knowledge, this is the first report of relationships between changes in selected OJIP-based parameters, and total FA (TFA) accumulation, a qualitative parameter of neutral lipid accumulation.

2. Materials and methods

2.1. Cultivation conditions

The Δ5 desaturase mutant of *P. incisa*, P127 [14] was obtained in the Microalgal Biotechnology Laboratory, J. Blaustein Institutes for Desert Research, and was cultivated on BG-11 medium [17] in 1 L glass columns (6 cm ID) under constant illumination (by daylight fluorescent lamps) of three different intensities (35, 130, and 270 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR as measured in the center of the empty column) and with constant bubbling of CO_2:air mixture (1:99, v/v) at 25 °C. Prior to the nitrogen-starvation experiment, cultures were daily diluted to maintain logarithmic growth. In all cases, initial chlorophyll content and DW upon transferring to nitrogen-free medium were maintained at 30 $mg \cdot L^{-1}$ and 1 $g \cdot L^{-1}$, respectively, to prevent photodamage of the cultures at high irradiance [3]. Before inoculation of the columns, cells were washed three times with sterilized distillated water and resuspended in nitrogen-free BG-11. The sampling for determination of DW, TFA content, pigment composition, and fluorescence measurements was performed at d 0 and following the 3rd, 7th, 10th and 14th d of nitrogen deprivation.

2.2. Fatty acid analysis

Capillary gas-chromatography was used for fatty acid quantification; the analysis was performed according to Cohen et al. [18]. The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analyzed in duplicate.

2.3. Pigment extraction and analysis

In routine measurements total Chl and Car were extracted from P127 biomass with dimethyl sulfoxide (DMSO) for 5 min at 70 °C with 5 mL per *ca.* 3.5 mg DW. The pigment concentrations were determined in DMSO extracts spectrophotometrically with a Cary 50 Bio spectrophotometer (Varian, Walnut Creek, CA, USA) [3]. Individual Car were analyzed in whole cells, isolated thylakoids and oil bodies (OB) using HPLC according to earlier published protocols [19]. Pigments were identified and quantified using pure pigment standards (Sigma-Aldrich, St. Louis, MO, USA; Fluka, Taufkirchen, Germany).

2.4. CF measurements and treatment of the data

Induction curves of CF (OJIP curves) were recorded using a Fluorpen FP100s portable Pulse Amplitude Modulated fluorometer (Photon Systems Instruments, Drasov, Czech Republic) according to the manufacturer's protocol. To increase the signal to noise ratio and to prevent irregularities related with cell sedimentation in the course of measurements the cells were concentrated on glass fiber filters GF/F (Whatman, UK). Pilot experiments were carried out to ensure that deposition on GF/F filters does not affect the shape or amplitude of OJIP curve as well as to find a suitable period of dark adaptation of the microalgae. Under our experimental conditions, a dark adaptation of 15 min allowed the reliable recording of OJIP curves.

The analysis of the recorded OJIP curves was carried out according to Strasser et al. [6]; the employed JIP-test parameters are detailed in Table 1 and in Fig. 1. Maximal quantum yield of PS II photochemistry, ϕ_{P_0} [10], and coefficient of non-photochemical Chl fluorescence quenching, NPQ [20], were also estimated using the FP100s fluorometer.

2.5. Fatty acid analysis

Capillary gas-chromatography was used for fatty acid quantification; the analysis was performed according to [18]. The data shown represent mean values with a range of less than 5% for major peaks (over 10% of fatty acids) and 10% for minor peaks, of at least two independent samples, each analyzed in duplicate.

2.6. Statistical treatment

The experiments were carried out in two biological replications with three analytical replications for each of them. In figures average