Short-term responses of primary processes in PS II to low temperature are sensitively indicated by fast chlorophyll fluorescence kinetics in Antarctic lichen Dermatocarpon polyphyllizum

Michaela Marečková, Miloš Barták*

Laboratory of Photosynthetic Processes, Section of Plant Physiology and Anatomy, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic

Abstract
In this study, we investigated the effects of low temperature on the fast chlorophyll fluorescence transient (OJIP) and OJIP-derived parameters in chlorolichen Dermatocarpon polyphyllizum exposed to a gradually decreasing temperature (22°C, 18°C, 14°C, 12°C, 10°C, 7°C and 4°C). The segments of lichen thalli were exposed to a certain temperature either in dark- and light-adapted state for 10 minutes in order to evaluate the effects on chlorophyll fluorescence parameters. The initial photochemical phase of the transient (O-J) due to reduction of the primary quinone acceptor (QA) was found temperature dependent. The K-step was apparent for the samples measured at the temperature above 12°C, but not below 10°C in light-adapted lichen thalli. With the thallus temperature decrease, majority of the chlorophyll fluorescence parameters derived from OJIP (ET0/RC, Psi_0, and DI0/RC) showed no change in light-adapted samples but a decrease in dark-adapted samples. The effects of dark-/light-adaptation of the lichen samples on the OJIP and OJIP-derived parameters was attributed to the differences in production/utilization of high-energy products of primary photochemical processes of photosynthesis in dark- and light-adapted state, respectively. The other parameters (ABS/RC, TR0/RC) showed a decrease with thallus temperature decrease both in light- and dark-adapted samples. The results suggest that fast chlorophyll fluorescence transient is an useful tool to investigate temperature-dependent changes in photosystem II in chlorolichens, their photobionts, respectively.

Key words: Chlorophyll fluorescence, fast kinetics, OJIP, K-step, James Ross Island, temperature stress

DOI: 10.5817/CPR2017-1-8


Received June 8, 2017, accepted July 18, 2017.
*Corresponding author: M. Barták <mbartak@sci.muni.cz>

Acknowledgements: The authors are grateful to the CzechPolar2 infrastructure that enabled sample collection and handling. Experimental part of work has been done in the EEL laboratory and supported by the ECOPOLARIS project (CZ.02.1.01/0.0/0.0/ 16_013/0001708).
level (in ms), PS I, PS II – photosystem I, II, QA – primary quinone acceptor, RC – reaction centre, TR0/RC – the trapped energy flux per reaction centre, Psi_0 – the probability that a trapped excitation moves an electron into the electron transport chain beyond QA.

Introduction

The fast chlorophyll fluorescence induction is defined as an increase of fluorescence emitted during the first two seconds of light exposure from predarkened and exposed sample of a photosynthetizing organism. In literature, the fast chlorophyll fluorescence kinetics are also abbreviated OJIP curves. General shape of OJIP curve is similar in all plant species. The O phase is an initial fluorescence (reaction centers are fully oxidised after dark adaptation). J and I are inflection points, this phase is related to the reduction of plastoquinone pool by reduced QA (Ilík et al. 2006). O to J rise is also called photochemical phase and it is not so sensitive to the temperature. Although it is dependent on amount of absorbed photons (Strasser et al. 1995, Stirbet et al. 2014). The peak P is maximum chlorophyll fluorescence. J-I-P rise is called thermal phase and more sensitive to the temperature changes. The shape of OJIP can be affected by several factors. Majority of them relate to the efficiency of excitation energy transport in PS II, the state of oxygen evolving complex and other processes involved in photosynthetic apparatus. In highly stressed samples, another inflection point K was reported between steps O and J (0.2 - 0.3 ms of chlorophyll fluorescence induction), indicating a disruption of oxygen-evolving complexes (Oukarroum et al. 2007, Xue et al. 2011).

Fast chlorophyll fluorescence measurement is a useful tool for non-invasive detection of various stress effects, such as e.g. high temperature (Brestič et al. 2013, Martinazzo et al. 2012), low and freezing temperature (Mishra et al. 2015), drought (Oukarroum et al. 2007, Brestič et Živčák 2013) or heavy metal soil contamination (Žurek et al. 2014). The chlorophyll fluorescence parameters change with different stresses. High temperature causes an increase of initial fluorescence F0 due to a dislocation of light-harvesting complexes II from PS II complexes, inactivation of PS II photochemical reaction or inhibition of electron flow caused by reduced transfer of QA to QB (Mathur et al. 2011). The decrease of FM in high temperature may be related to denaturation of chlorophyll proteins (Yamane et al. 1997). Occurrence of K-step indicates heat stress of a plant and it is related to the oxygen-evolving complex disruption. Drought stress affects PS II apparatus only under extreme conditions - it has a higher resistance to water deficiency than PS I (Brestič et Živčák 2013). Salinity stress is often associated with a decrease of photosynthetic capacity of plants. Salinity strongly limits photosynthetic CO2 fixation, light energy absorption by photosynthetic pigments is higher than its consumption in chloroplasts and excess energy may accelerate the photodamage to PS II by generating of reactive oxygen species. The decrease of maximum quantum yield of PS II and an increase of non-photochemical quenching duo to high salinity have been recorded (Yang et al. 2007, He et al. 2009). Deficiency in specific nutrients (such as N, K, P, Ca, Mg, S or Fe) also disrupts the functioning of the photosynthetic apparatus. It decreases PS II photochemical efficiency and modifies the values of chlorophyll fluorescence parameters (Smethurst et al. 2005). Under the low temperature stress a variable chlorophyll fluorescence is decreased as well as photochemical quenching and efficiency of open PS II reaction centers (Yang et al. 2007, Mishra et al. 2015). Non-photochemical quenching of absorbed excitation energy increases
with decreasing temperature. The net photosynthesis decreases with the temperature due to low supply of ATP and NADPH from temperature-dependent limitation of photochemical processes. These are dependent on electron transport through temperature-sensitive thylacoid membrane.

In lichens, OJIP is used to evaluate the responses of thalli to rehydration at different temperatures (Oukarroum et al. 2012, Barták et al. 2015b), photoinhibition (Balárinová et al. 2014), ultraviolet radiation (Medina et Avalos-Chacon 2015), and fast reoxidation of PS I (Ilík et al. 2006). In this study, we applied fast chlorophyll fluorescence transients to evaluate the response of Dermatocarpon polyphyllizum, an Antarctic chlorolichen, to a short-term decrease in thallus temperature. We hypothesized that the early events in the absorption and transfer of energy in photosystem II of a symbiotic alga, energy fluxes and effectiveness of energy transfer in particular, would be temperature-dependent.

Material and Methods

Species characteristics

Dermatocarpon polyphyllizum is a lichen species, that belongs to the lichen family Verrucariaceae. It usually grows on the rocks in higher altitudes or in polar regions. D. polyphyllizum has a dark brown foliose thallus. Recently, a very little is known about biosymbiont species of Dermatocarpon, although green alga Diplosphaera chodatti (Trebuxiophyceae) was identified in few members of this genus (Fontaine et al. 2012). Upper cortex is 60-120 µm thick with attached clavate or cylindrical asci with 8-spored ascospores (Nash et al. 2004).

Sample collection and handling

Samples of D. polyphyllizum lichen were collected on James Ross Island in February 2017 from the long-term research plot (LTRP, James Ross Island, Antarctica – for the sampling site description, see Barták et al. 2015a). Then, the samples were stored dry at 10°C. All samples of dry thalli were allowed to rehydrate for at least 48 h before an experiment at the temperature of 10°C while exposed to the light.

Chlorophyll fluorescence measurements

OJIP curves: Rehydrated samples of Dermatocarpon polyphyllizum were stored at 4°C. Round discs were cut from the samples, then upper cortex was carefully removed. This uncovered photobiont layer and allowed higher chlorophyll fluorescence (ChlF) signals. A small piece of square-shaped filter paper was put under the disc to moist the samples. Three discs of Dermatocarpon were placed into a Petri dish with demineralised water. The Petri dish was placed into the cooling unit and aclimated for 10 min.

OJIP curves were measured using FluorPen fluorometer (Photon Systems Instrumens, Drásov, Czech republic) in particular temperatures (T = 22°C, 18°C, 14°C, 12°C, 10°C, 7°C and 4°C) with 10
min. acclimation at each temperature. Temperature was measured during whole experiment by hairy Cu-Co thermocouple in 30 s interval and stored into a data logger (Edge Box, Environmental Measuring Systems, Brno, Czech Republic). Actual temperature of discs was also observed on-line through a EMS software. The first set of experiments was with the lichen samples (thallus segments) darkened during whole cooling experiment (DA – dark adapted). In the second set of experiments samples were predarkened only 5 min. before every measuring and illuminated in between (LA – light adapted).

For visualisation of K-step the data of ChlF transients were doubly normalised between \( F_0 \) and \( F_J \) (2 ms), expressed as \( W_{01} = (F_J - F_0)/(F_J - F_0) \). The differential transients enables visualisation of K-step at 0.25-0.3 ms (see Fig. 3). Reference temperature effects on photosynthetic performance of \( D. \) polyphyllizum, the below-listed chlorophyll fluorescence parameters were derived from the data taken during the OJIPs measurements according to the equations of Strasser et al. (2000). The parameters have the following meanings: \( \Phi_P^\text{av} \) is the time to reach maximum chlorophyll fluorescence level (in ms), \( \text{ABS/RC} \) is absorbed energy per reaction centre (RC), \( \text{ET}_\text{RC} \) is electron transport flux per RC, \( \text{TR}_\text{RC} \) is the trapped energy flux per RC, \( \Psi_0 \) is the probability that a trapped excitation moves an electron into the electron transport chain beyond \( Q_A \), \( \text{DI}_\text{RC} \) is dissipated energy flux per RC.

\[
\begin{align*}
\Phi_P^\text{av} &= \Phi_P^0 \cdot (S_M/t_{FM}) \\
\text{ABS/RC} &= M_0 \cdot (1/V_J) \cdot (1/\Phi_P^0) \\
\text{ET}_\text{RC} &= M_0 \cdot (1/V_J) \cdot \Psi_0 \\
\text{TR}_\text{RC} &= M_0 \cdot (1/V_J) \\
\Psi_0 &= \text{ET}_\text{RC}/\text{TR}_\text{RC} = 1 - V_J \\
\text{DI}_\text{RC} &= (\text{ABS/RC}) - (\text{TR}_\text{RC})
\end{align*}
\]

**Results and Discussion**

Shape of OJIP curves was found temperature-dependent in \( D. \) polyphyllizum. In the dark-treated samples, J step increased with thallus temperature decrease. In light-treated samples, however, the thallus temperature decrease brought an overall decrease of chlorophyll fluorescence signal throughout the whole polyphasic stansent from O to P. The more pronounced decrease in thallus temperature, the lower values of chlorophyll fluorescence were apparent. Both in dark- and light-treated samples, the decrease in thallus temperature led to a shift in the time at which the P level of chlorophyll fluorescence was reached. It was 0.41 / 0.42 s at 22°C (dark-/light-treated samples). For the samples exposed to 4°C, the time was 0.93 / 0.65 s, respectively. After the P peak, a decrease in chlorophyll fluorescence was seen irrespective of the treatment and experimental temperature. However, no H peak peak as reported (Ilík et al. 2006) for chlorolichens and their dominant algal symbiont (\( Trebouxia \) sp.) was seen. The presence of H peak is attributed to ferredoxin-NADP⁺ reductase (FNR) activation (e.g. Stirbet et al. 2014) in algal photobionts of chlorolichens. The reason for the absence of H peak seems to be unclear, however, might be associated with intrinsic properties of symbiotic alga in \( D. \) polyphyllizum.
In the Verrucariaceae family, a high diversity of algal symbiont is seen, including some algal species that are rarely or never associated with other lichens (Thüs et al. 2011). In *D. miniatum*, the photobiont was identified as *Hyalococcus dermatocarponis* (Fontaine et al. 2012). Contrastingly, an amphibious species (*D. luridum*) and a species from exposed rock surfaces (*D. miniatum*) have *Diplosphaera* as a photobiont (Thüs et al. 2011). Similarly, Řeháková (1968) reported *Diplosphaera chodatii* as the photobiont of several *Dermatocarpon* species. Since photosynthetic properties of the above-mentioned algal species are mostly unknown, we may only hypothesize that the presence/absence of H peak in lichens is related to interspecific differences in photosynthetic apparatus of the symbiotic alga, *i.e.* between genus *Trebouxia* (H peak present), and genus *Diplosphaera* (H peak absent or undetectable).

**Fig. 1.** Thallus of *Dermatocarpon polyphylizzum* (left) and cross section (right) with the indication of the upper cortex (a), photobiont layer (b), peritheciurn (c), and lower cortex (d). Photos by M. Marečková.

**Fig. 2.** Fast chlorophyll fluorescence transients (OJIPs) as related to thallus temperature measured on dark-adapted (left), and light-adapted samples (right). The OJIPs were measured on thallus segments with the upper cortex scraped. To document the effect of upper cortex on the attenuation of chlorophyll fluorescence signal, a single thallus (indicated by an asterisk) was measured with the upper cortex.
Fig. 3. Differential plot of the initial part of OJIP - W_{OJ} (double-normalized) in response to experimental temperature. The curves were measured in dark-adapted state (left), and light-adapted state (right).

In double-normalized differential plot of the initial part of OJIPs, the K-step was found. K-step appeared at 22°C at 0.21 ms in *D. polyphyllizum* treated in light. In the dark-treated samples exposed to different temperature, however, the K-step was seen also at 12°C (0.34 ms), and 14°C (0.29 ms), respectively (see Fig. 3). It indicates that the negative effect of the combination of factors (temperature and light) on the appearance of the K-step is more pronounced than for temperature *per se*. In spite of relatively low light dose (32 μmol m⁻² s⁻¹), the inhibitory effect of high temperature (22°C) on oxygen evolving complex (OEC) was higher than in dark-treated samples.

It is well established that the K step occurring at around 0.30 ms is caused by an inhibition of the OEC (see e.g. Guissé et al. 1995, Srivastava et Strasser 1996). In this concept, the K-step reflects an imbalance between the electron flow leaving the RC towards the acceptor side and the electron flow coming to the RC from the donor side. Such situation is associated with the accumulation of a high fluorescence yield species (speculated to be the pheophytin: Pheo). Therefore, the amplitude of step K is used as a specific indicator of damage to PS II donor side frequently (see e.g. Hendrickson et al. 2003), especially in plant exposed to heat stress (Chen et al. 2016). Our data (see Fig. 3) suggest that donor side inhibition may occur in *D. polyphyllizum* at the temperature higher than 16°C. However, another explanation might be also possible.

The effect of dark- / light-treatment on numeric values of the OJIP-derived parameters is shown in Fig. 4. If the parameters are measured in dark-adapted sample, the effect might be positive (Phi_Pav), however, negative effect is apparent in a majority of parameters (ET_0/RC, Psi_0, DI_0/RC, ABS/RC, and TR_0/RC). Therefore, a positive role of light on numeric values of the OJIP-derived parameters measured in response to temperature might be considered. In general, the effects of dark- light-treatment on OJIP and OJIP-derived parameters is associated with the production/utilization of high-energy products of primary photochemical processes of photosynthesis. In the samples kept in the darkness, the connection between the light driven electron transfer (the production of NADPH and ATP), and the carbohydrate synthesis, through the Calvin–Benson cycle, is severed (for a review see Stirbet et Govindjee 2012).
The reduction of NADP⁺ by reduced ferredoxin is catalyzed by FNR. In samples that had been in dark, FNR must be activated, a process that in higher plants takes several seconds, but algae, lichen and corals need only a few 100 ms (see e.g. Schansker et al. 2008). Inactivation of FNR in the darkness is plant species-dependent. It is completed within 15 min. in pea leaves (Schansker et al. 2006) and it may take as much as an hour in conifers. Therefore, we may attribute the decrease in numeric values of the OJIP-derived parameters in dark-treated samples (Fig. 4) to an imbalance between photochemical and biochemical processes of photosynthesis caused by dark-treatment lasting tens of minutes in this study.
References


LICHEN PHOTOSYNTHESIS AT LOW TEMPERATURE


