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Photosynthesis of lichen symbiotic alga *Trebouxia erici* as affected by irradiance and osmotic stress

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Abstract

The relation between oxygen evolution rate (OER) and quantum yield of photochemical reactions in photosystem 2 (Φ_{PS2}) was examined in lichen symbiotic alga *Trebouxia erici* Ahmadjian (strain UTEX 911) exposed to different irradiances and osmotic stress (2 M sucrose for 60 h). Linear relationship was found between OER and Φ_{PS2} in control cell suspension within irradiance range of 0 - 500 μmol m^{-2} s^{-1}. Under osmotic stress, OER and Φ_{PS2} were significantly reduced. Relation between OER and Φ_{PS2} was curvilinear due to strong osmotically-induced inhibition of OER at high irradiance. The highest used irradiance (500 μmol m^{-2} s^{-1}) was photoinhibitory for osmotically-stressed *T. erici* because non-photochemical quenching (NPQ) increased substantially. Energy-dependent quenching represented major part of NPQ increase. Osmotic stress led also to the reduction of capacity of photochemical processes in PS 2 (F_V/F_M) and increase in F_0/F_M. These changes indicated negative effects of osmoticum on structure and function of photosynthetic apparatus.

Additional key words: chlorophyll fluorescence, oxygen evolution, photobiont, photosynthesis, quantum yield.

Introduction

In lichens possessing green alga as symbiotic photobiont, algal genuses *Trebouxia*, *Trentepohlia*, and *Coccomyxa* are the most abundant (Friedl and Büdel 1996). Among them, *Trebouxia* sp. has been studied frequently mainly with respect to its phylogeny and association with mycobiont forming lichens of different genuses (*e.g.* Romeike *et al.* 2002, Opanowicz and Grube 2004). Within last few decades, physiological characteristics of *Trebouxia* sp. have been investigated at the level of whole thallus mainly in relation to photosynthetic pigments pools (Czeczuga *et al.* 2004), capacity of photosynthetic production of polyols, and photobiont-to-mycobiont transport polyols (Dahlman *et al.* 2003). On isolated algal cells and *in vitro* cultured populations of *Trebouxia*, the impact of heavy metal toxicity has been investigated (Bačkor *et al.* 1998, Bačkor and Váczi 2002, Bačkor and Dzubaj 2004) in last decade. Photosynthetic activity of green-algal and cyanobacterial lichen photobionts was studied by Palmqvist (1993), Palmqvist *et al.* (1997, 1998), and Smith and Griffiths (1998). These studies focused mainly on the role of carbon concentrating mechanism in net photosynthetic rate of *Trebouxia* cells in response to irradiance and CO_2 availability. Despite the above-cited studies, information is still scarce on the relation of photochemical and biochemical processes of photosynthesis in *Trebouxia* sp. Moreover, knowledge on photosynthesis in *Trebouxia* sp. under different stresses is almost lacking.

At the level of whole lichen thallus, however, the effect of different stresses on net photosynthetic rate (P_N) has often been studied *in situ* (Renhorn *et al.* 1997, Lange 2002, 2003a,b,). Effect of dehydration of a lichen thallus on P_N has been studied by Heber *et al.* (2000), Mackenzie and Campbell (2001), Rascher *et al.* (2003), *etc.*

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Abbreviations: Chl - chlorophyll; LHC 2 - light harvesting complex of PS 2; NPQ - non-photochemical quenching; OER - oxygen evolution rate; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density; PS 2 - photosystem 2; q_E - energy-dependent quenching; Φ_{CO2} - quantum yield of photosynthetic CO_2 fixation; Φ_{PS2} - quantum yield of photochemical processes in PS 2; ψ - water potential

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Some studies on lichens investigated the response of photosynthesis to dehydration using induced osmotic stress (Jensen *et al.* 1999) because physiological consequences of osmotic stress are similar to atmospheric dehydration (Calatayud *et al.* 1997). Photobionts in lichen thalli reduce P_N with increasing osmotic stress (Chakir and Jensen 1999) and exhibit slow but still detectable photochemical processes of photosynthesis, quantum yield of PS 2 (Φ_{PS2}) in particular, even under extremely low water potential (Ψ from -15 to -30 MPa; Barták *et al.* 2005, Hájek *et al.* 2005).

We focused on the effect of osmotic stress (mimicking the extent of dehydration stress in desiccating lichen thallus) induced under laboratory conditions on primary photosynthetic processes in *Trebouxia erici*. We hypothesized that, similarly to results presented by Chakir and Jensen (1999), increasing osmotic stress would lead to a decrease in Φ_{PS2}. Moreover, we investigated the relation between photochemical and biochemical processes of photosynthesis, photosynthetic oxygen evolution rate (OER) in particular. We hypothesized that, the relation between Φ_{PS2} and OER would be linear under physiological conditions. Under osmotic stress mimicking dehydration of *Trebouxia* cells, however, we expected deviations of the relationship from linearity because there is an experimental evidence from a field study (Green *et al.* 1998) that the relation between Φ_{PS2} and apparent quantum yield of photosynthesis (Φ_{CO2}) is curvilinear in green algal lichen *Umbilicaria aprina.*

Materials and methods

Photobiont cultivation: Stock cultures of the lichen photobiont *Trebouxia erici* Ahmadjian (Fig. 1) were maintained in an axenic culture (UTEX 911) on Bold's Basal Medium (BBM-agar) in Petri dishes at temperature of 20 °C and 16-h photoperiod with irradiance of 30 μmol m^{-2} s^{-1}. The medium prepared according to Ahmadjian (1993) contained per dm^3: 0.25 g $NaNO_3$ (0.75 g $NaNO_3$ in BBM3N medium); 0.175 g KH_2PO_4; 0.075 g K_2HPO_4; 0.075 g $MgSO_4.7\ H_2O$; 0.025 g $CaCl_2$; and 0.025 g NaCl. In addition, 1 dm^3 of medium contained the following micronutrients: 11.42 mg H_3BO_3; 4.95 mg $FeSO_4.7\ H_2O$; 8.82 mg $ZnSO_4.7\ H_2O$; 1.44 mg $MnCl_2.4\ H_2O$; 0.71 mg MoO_3; 1.57 mg $CuSO_4.5\ H_2O$; 0.49 mg $Co(NO_3)_2.6\ H_2O$; 50 mg EDTA, and 31 mg KOH. After 2-month cultivation on BBM agar medium, algal cultures were transferred into a liquid BBM3N medium (Ahmadjian 1993), suspended by gentle stirring on a magnetic stirrer for 1 h.

Osmotic stress induction: Five-day-old stock culture suspensions were transferred into Erlenmeyer flasks (100 cm^3) and culture density was adjusted by adding the sterilized medium to a constant value (absorbance at 750 nm: 0.8). To induce osmotic stress, sucrose was added into a flask to reach concentration of 2 M (water potential ψ = -11.35 MPa). To determine steady state of photosynthetic parameters in osmotically-treated cell suspension, Φ_{PS2} was measured repeatedly by a modulated fluorometer *OS-FL1* (*OptiScience*, Tyngsboro, USA) until it reached constant value (after 60 h).

Simultaneous measurement of Φ_{PS2} and OER: For determination of Φ_{PS2} of *T. erici* suspension, a double-modulated fluorometer *FL-200* (*Photon Systems Instruments*, Brno, Czech Republic) with measuring compartment containing a 3 cm^3 exposure cuvette was used. The cuvette was equipped with an in-built oxygen electrode *OC 223-B* (*THETA '90*, Praha, Czech Republic) connected to a computer-aided O_2-meter (*OxyCorder 401*, *Photon Systems Instruments*). The temperature of the suspension in the cuvette was maintained constant (24 °C) throughout all measurements by a thermo-regulator *TR2000* (*Photon Systems Instruments*). Temperature was measured by needle temperature sensors (copper-constantan thermocouples, 0.45 mm in diameter) and recorded by a data logger *MiniCube* (*EMS*, Brno, Czech Republic). The photobiont suspension was continuously stirred in the exposure cuvette by a magnetic stirrer. Within the measurement protocol, the photobiont suspension was exposed to a stepwise increased irradiance (red LED diodes; λ_{max} = 615 nm). Photo-synthetic photon flux densities (PPFD) were 37, 66, 135, 270, and 500 μmol m^{-2} s^{-1} and at the end of each 10 min-lasting irradiation, saturation pulse was applied on this light-adapted suspension (orange LED diodes; λ_{max} = 626 nm). Steady-state chlorophyll (Chl) fluorescence (F_S) before saturation pulse and maximum value of Chl fluorescence (F_M') induced by the saturation pulse were recorded and used for Φ_{PS2} calculation (see Table 1). Simultaneously, O_2 concentration in photobiont suspension was measured and recorded in 1 s interval during the exposure to each PPFD. OER was assessed as a mean slope of equilibrated O_2 concentration rise at each PPFD. Finally, Φ_{PS2} and OER were related to each other and expressed as dependent on PPFD. The above procedure was applied for untreated (control) and osmotically-stressed photobiont suspensions.

To characterize the PPFD response curve of photosynthesis in *T. erici* suspension, OER data were plotted against irradiance and fitted by an exponential (Potvin *et al.* 1990). Dark respiration was evaluated as a negative OER derived from the equation of the best fit when irradiance (input parameter) was set zero. Maximum OER was derived as an asymptotic value of the fit in high irradiance.

Chl fluorescence parameters in response to osmotic stress: A set of Chl fluorescence parameters was

measured on control and osmotically-stressed samples after 60 h incubation. Basic parameters (F_V/F_M, F_0/F_M, Φ_{PS2}, NPQ) were determined from analysis of recorded kinetics of Chl fluorescence according to the following measurement protocol. On dark-adapted (sufficient time of 10 min was tested before) samples, weak irradiance was applied in order to determine background Chl fluorescence (F_0). Then a saturation pulse allowed to calculate maximum capacity of PS 2 (F_V/F_M). Steady-state Chl fluorescence (F_S) and maximum Chl fluorescence on samples adapted to PPFD provided by the *FL-200* fluorometer (F_M') were determined (as described above) for all applied PPFDs. The light was then turned off and a final saturation pulse (the peak was denoted as F_M'') was applied after 10 min of dark relaxation. Subsequently, components of Chl fluorescence quenching, *i.e.* energy-dependent quenching (q_E) and state-transition and photoinhibitory quenching (q_{T+I}), were determined for the highest used PPFD (500 μmol m^{-2} s^{-1}).

Results

Photosynthesis in response to irradiance: With increasing irradiance, quantum yield of PS 2 (Φ_{PS2}) decreased from 0.38 recorded in dark to 0.15 recorded at 500 μmol m^{-2} s^{-1} (Fig. 2) in control suspension of *Trebouxia erici* culture. OER showed curvilinear increase with increasing irradiance reaching its maximum 5.06 pg(O_2) (1000 cells)$^{-1}$ s^{-1} at about 500 μmol m^{-2} s^{-1} (Fig. 2). In osmotically-stressed suspension, however, OER and Φ_{PS2} were significantly reduced (Fig. 2 - 2 M sucrose). Φ_{II} exhibited an exponential decrease with increasing irradiance with plateau at PPFD above 300 μmol m^{-2} s^{-1}. In osmotically-stressed cells, in contrast to control, the OER reached maximum [2.8 pg(O_2) (1000 cells)$^{-1}$ s^{-1}] at about 130 μmol m^{-2} s^{-1} and showed a photoinhibition-induced decrease with increasing irradiance reaching low constant value of 0.04 pg(O_2) (1000 cells)$^{-1}$ s^{-1} at about 300 μmol m^{-2} s^{-1}.

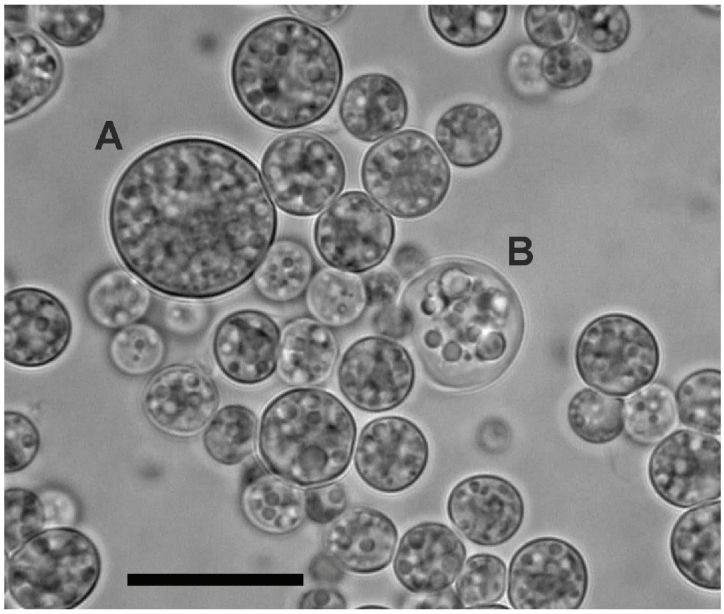

Fig. 1. Cell suspension of *Trebouxia erici* cultivated in BBM medium. *A* - large vegetative mature cell with finished volume growth, *B* - ruptured cell with aplanospores. *Scale bar* represents 5 μm.

Relation of Φ_{PS2} to OER studied simultaneously under a stepwise increased irradiance (Fig. 3) was fairly linear in the control, showing typically decreasing trend of OER towards high Φ_{PS2}. Maximum recorded OER was 5.06 pg(O_2) (1000 cells)$^{-1}$ s^{-1} at 500 μmol m^{-2} s^{-1}. Φ_{PS2} at the same irradiance reached the lowest value of 0.17. In dark, respiration processes in *T. erici* cell suspension resulted in a negative OER [-3.76 pg(O_2) (1000 cells)$^{-1}$ s^{-1}]. In dark, maximum Φ_{PS2} reached the value of 0.32. In an osmotically-stressed cell suspension, OER values were allways lower for a given Φ_{PS2} than in control suspension (Fig. 3). The decrease in OER was significant in whole Φ_{PS2} range. In contrast to control, osmotically-stressed cell suspension exhibited curvilinear relationship of OER to Φ_{PS2}. Maximum deviation of the relationship from linearity was found under conditions typical by lowest and highest Φ_{PS2}, *i.e.* high irradiance and darkness. In the dark, due to a substantial proportion of respiration, OER

was more negative [-6.34 pg(O_2) (1000 cells)$^{-1}$ s^{-1}] than control. With increasing PPFD, and thus decreasing Φ_{PS2}, OER increased until Φ_{PS2} of 0.1, and then OER slightly decreased reaching other minimum (Fig. 3). At high PPFD, OER reached zero and Φ_{PS2} was still detectable (Φ_{PS2} = 0.05).

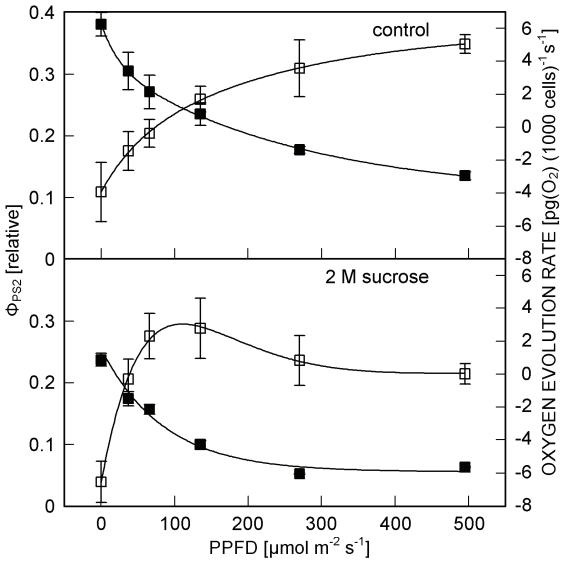

Fig. 2. Relation of quantum yield of photochemical reactions in photosystem 2 [Φ_{PS2} - *closed squares*] and oxygen evolution rate [OER - *open squares*] to PPFD in control and osmotically stressed (2 M sucrose) *Trebouxia erici* cell suspension. The *bars* indicate ± SD. Means are of at least 5 replicates.

Osmotic effects on Chl fluorescence parameters: A 60 h exposure of *T. erici* cell suspension to 2 M sucrose led to changes in Chl fluorescence parameters (Table 1). Maximum capacity of photochemical processes in PS 2 (F_V/F_M) was reduced to 35 % and the reduction of quantum yield of PS 2 reached 72 % of the pre-exposure value. Exposure to 2 M sucrose increased utilization of absorbed photon energy in non-photochemical processes, energy-dependent quenching of Chl fluorescence (q_E) in particular. Non-photochemical quenching (NPQ) was about 2.5 fold higher than in control, q_E increased by about 9 times. The other component of NPQ, q_{T+I} which is related to photoinhibitory and state-transition processes in photosynthetic apparatus, remained unchanged. Osmotic stress induced 23 % increase in F_0/F_M which indicated structural changes in peripheral LHCs, their detachment from PS 2 core, respectively.

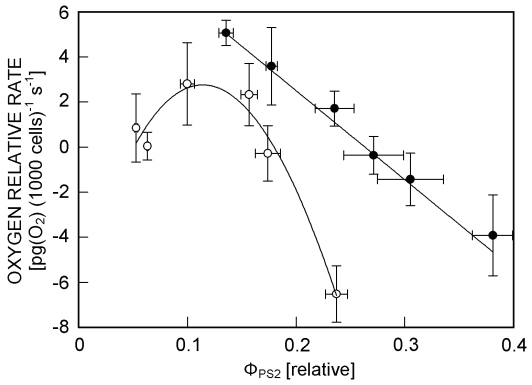

Fig. 3. Oxygen evolution rate (OER) in relation to quantum yield of photochemical reactions in photosystem 2 (Φ_{PS2}) at a stepwise increasing PPFD for *Trebouxia erici* cell suspension in control (*closed circles*) and under osmotic stress (*open circles*). The *bars* indicate ± SD of OER (*vertical bars*) and Φ_{PS2} (*horizontal bars*), respectively. Means are of at least 5 replicates.

Table 1. Chlorophyll fluorescence and gas exchange parameters measured in control and osmotically stressed *Trebouxia erici* cell suspension after 60 h incubation. Φ_{PS2} was measured under PPFD of 500 μmol m^{-2} s^{-1}. Means ± SE, * - statistically significant difference tested by a Student *t*-test at $P < 0.001$. F_M - maximum fluorescence on dark-adapted sample, F_M' - maximum fluorescence on light adapted sample (under irradiance), F_M'' - maximum fluorescence measured 10 min after turning off the radiation, F_0 - background fluorescence, F_0' - background fluorescence during radiation (determined as Chl fluorescence level measured immediately after turning off the light), F_S - steady-state fluorescence during irradiation. For calculations of quenching coefficients in which F_M is an input parameter, pre-photoinhibition F_M values were used. The parameters were compiled from Genty *et al.* (1989), Van Kooten and Snel (1990), Roháček and Barták (1999), and Schreiber *et al.* (1995a).

Chl fluorescence parameter	Definition	Control	2 M sucrose
Maximum capacity of PS 2 (Fv/F_M)	F_V/F_M	0.381 ± 0.0192	0.257 ± 0.0126*
Quantum yield of photochem. reactions of PS 2, (Φ_{PS2})	$(F_M' - F_S)/F_M'$	0.151 ± 0.0072	0.043 ± 0.0059*
Non-photochemical quenching, (NPQ)	$(F_M - F_M')/ F_M'$	0.227 ± 0.0381	0.567 ± 0.0462*
State-transition +photoinhibitory quenching, (q_{T+I})	$(F_M - F_M'')/(F_M - F_0')$	0.350 ± 0.0604	0.365 ± 0.0354*
Energy-dependent quenching, (q_E)	$[1-(F_M' - F_0')/(F_M - F_0)] - q_{T+I}$	0.063 ± 0.0508	0.550 ± 0.0145*
Relative backround Chl fluorescence (F_0/F_M)	F_0/F_M	0.606 ± 0.0052	0.743 ± 0.0126*
OER_{max} [pg(O_2) (1000 cells)$^{-1}$ s^{-1}]		5.07	1.39
Dark respiration rate [pg(O_2) (1000 cells)$^{-1}$ s^{-1}]		3.76	6.34

Discussion

Decrease in Φ_{PS2} found in osmotically-treated cell suspension of *Trebouxia erici* is a general response of algal photosynthesis to osmotic stress. In algae, similar response is reported for a variety of stressors, such as high irradiance (Ritz *et al.* 1999), high temperature (Sayed and El-Shahed 2000), limited nitrogen supply (Sayed 1998) and heavy metals (Küpper *et al.* 2003), *etc*. Osmotic treatment led to F_0/F_M increase in *T. erici*. That is again considered as an indicator of general stress at the level of LHC 2 that might be induced by different stressors; *e.g.* temperature stress (Lovelock *et al.* 1995), osmotic stress (Hájek *et al.* 2005), and high irradiance (Bertamini and Nedunchezhian 2004). In our experiment, osmotically-induced decrease in Φ_{PS2} in *T. erici* indicated decline of the rate of photosynthetic electron transport in thylakoid membranes and increased involvement of non-photochemical pathways of dissipation of absorbed radiation energy. Since Φ_{PS2} decrease was apparent throughout irradiances in this study, osmotic stress might lead to inhibition of photochemical processes in PS 2 of *T. erici* under all environmental irradiances. When compared to experimental evidence (Hájek *et al.* 2005) reached on whole lichen thallus with symbiotic *Trebouxia* sp. treated with sucrose under similar conditions (thallus temperature of 22 °C), our data exhibited slightly higher extent of Φ_{PS2} reduction than that reached by the same osmotic concentration added to whole thallus. This might be explained by a buffering effect of hyphal cortex layers of a lichen thallus. Thus, effective concentration of osmotically-active compound might be lower in algal layer within a lichen thallus then in algal cell suspension exposed to osmoticum directly. Therefore, negative effects of osmotically-active compound might be less pronounced in a thallus than in cell suspension. *Trebouxia*, however, is resistant to osmotic stress. When re-calculated to water potential, concentration of sucrose gives ψ of -11.35 MPa. This represents only moderate water stress in poikilohydric lichens. Barták *et al.* (2005) showed that lichen thalli exposed to atmospheric dehydration exhibited only small decrease below maximal Φ_{PS2} in the Ψ range of 0 to -10 MPa, while Ψ below -10 MPa induced substantial decrease in Φ_{PS2}. Similarly, Chakir and Jensen (1999) reported that Ψ below -8 MPa is required for a decrease in Φ_{PS2} in sucrose- and NaCl-treated lichen *Lobaria pulmonaria*. Another example of low Ψ required for Φ_{PS2} reduction was given by Kawamitsu *et al.* (2000) who considered water potentials below -10 MPa as critical for photosynthesis expressed as OER in sea inter-tidal alga *Fucus vesiculosus.*

Our results suggest that in non-stressed algal cell suspension, OER is well linearly related to Φ_{PS2}. Under osmotic stress, curvilinear relation of Φ_{PS2} to OER and much lower OER (compared to control) were recorded which might be attributed to reduced photosynthetic CO_2 fixation in stressed *T. erici* cell suspension, caused by increased photorespiration rate. Especially at high PPFD (500 μmol m^{-2} s^{-1}) osmotic stress led to severe photoinhibition in *T. erici* exhibiting OER of 0.04 pg(O_2) (1000 cells)$^{-1}$ s^{-1}. Combined negative effects of osmotic and high irradiance stress on photosynthesis are common in algae, even in osmotic-tollerant seagrass species (Ralph 1999). Reduced photosynthesis is a common phenomenon in poikilohydric lichens, symbiotic algae in particular, that are subjected to dehydration below optimum water content in a thallus (*e.g.* Lange 1988, 2002, Sundberg *et al.* 1997). Negative physiological and photosynthetic effects induced in lichen thallus by atmospheric dehydration and osmotic stress are probably similar (Chakir and Jensen 1999). Osmotically-induced inhibition of P_N might be explained as a result of decreased rate of photo- and biochemical processes under such conditions (Jensen *et al.* 1999). In poikilohydric lichens, when the water content declines below the optimum for assimilation, photosynthetic activity is inhibited as a consequence of dehydration (desiccation) effects on metabolic processes of photobiont. Under severe dehydration, cell structure of photobiont (alga or cyanobacterium) is strongly affected mainly by protoplast contraction due to loss of water and changes in chloroplast shape. Both atmospheric dehydration and/or osmotically-induced dehydration increase probability of irradiance injuries to PS 2. In dehydrated state, less absorbed energy is transported trough PS 2 and excess energy might cause damages to PS 2 structure and function. These alterations lead to the reduction of P_N, Φ_{PS2} in particular. To prevent damage to PS 2, several protective mechanisms promoting non-photochemical utilization of excess energy (expressed as an increase in NPQ) are activated in desiccating/dehydrating cells of poikilohydric plants (Deltoro *et al.* 1998). Simultaneously with NPQ rise, capacity of carotenoids and antioxidants to scavenge excitation energy from energized Chl molecules increases (Kranner 2002). Activation of such protective mechanisms reduces Φ_{PS2}, and thus also utilization of absorbed radiation energy in Calvin cycle. Another interacting mechanism, that reduces photosynthesis under osmotic stress, could be a size of internal pool of dissolved carbon (CO_2 + HCO_3^-) in photobiont cells that is available for photosynthetic processes (Palmqvist 1997). The smaller the pool is, the more reduced photosynthesis might be expected. It is reported for *Trebouxia*, that the amount of dissolved carbon is rather small compared with, *e.g.*, *Chlamydomonas reinhardtii* (Palmqvist 1997). However, actual amount of dissolved carbon in *Trebouxia* cells depends on the CO_2 transport rate and accumulation. In *Trebouxia*, capacity and exploitation of carbon concentrating mechanism in dehydrated or osmotically treated lichens might be another interacting factor affecting photosynthetic response (Palmqvist and

Sundberg 2000). Moreover, drop of Φ_{PS2} with osmotic dehydration might be more pronounced when there is another interacting stress factor. Nutrient availability in lichen thallus and/or green symbiotic algae might affect P_N and biomass production as shown *e.g.* for nitrogen (Dahlman *et al.* 2003) and phosphorus (Litchman *et al.* 2003).

In our study, Φ_{PS2} recorded in stepwise increasing radiation was linearly correlated to OER in non-stressed *T. erici* cell suspension. This indicated efficient transport of absorbed photon energy through photochemical and biochemical processes of photosynthesis. Linearity of the relation of Φ_{PS2} to biochemical processes (quantum yield of CO_2 fixation, Φ_{CO2}) is well documented in higher plants in response to increasing PPFD (Oberhuber *et al.* 1993). The relation is fairly linear over a wide range of PPFDs with small deviation from linearity at PPFD close to zero, when the rate of respiration represent substantial proportion of total CO_2 exchange (relative to gross photosynthesis). Thus in higher plants at low PPFD, Φ_{CO2} is somewhat lower than expected from linearity of Φ_{PS2} to Φ_{CO2} relation. At high PPFD, on the other hand, proportion of respiration to gross photosynthesis decreases.

In irradiated algae, slightly curvilinear relation of Φ_{PS2} to the yield of biochemical processes of photosynthesis is reported. Figueroa *et al.* (2003) showed slightly curvilinear Φ_{PS2} to Φ_{O2} relation for green macroalgae *Ulva rotundata* and *U. olivascence* and the red *Porphyra leucosticta* exposed to a wide range of PPFD. The slight non-linearity might be caused by heterogeneity of PS 2 in photosynthetic apparatus, *e.g.* Schreiber *et al.* (1995b) reported two populations of PS 2 showing different effectivity of electron transport in an eukaryotic alga. Our results for non-stressed *T. erici* are comparable to those of Figueroa *et al.* (2003), because if our data are re-calculated as Φ_{PS2}/Φ_{O2} relation (not shown here), the relation becomes slightly curvilinear.

Under osmotic stress, Φ_{PS2}/OER was curvilinear and for single Φ_{PS2}, somewhat lower values of OER were recorded than in control. Non-linearity of the relation caused by decreased OER values at high/low PPFD, *i.e.*, when low/high Φ_{PS2} are recorded, might be explained as a consequence of osmotically-induced decrease in P_N. Also, negative effect of high concentration of osmotically-active sucrose led to negative structural changes in chloroplasts and their components, similarly as shown by Stoynova-Bakalova and Toncheva-Panova (2003/4). Functioning of osmotically-affected chloroplasts was inhibited, resulting in apparent non-linearity of Φ_{PS2} to OER relation.

For lichens measured at whole-thallus level, non-linearity of Φ_{PS2}/Φ_{CO2} was reported by Green *et al.* (1998). Since hydration is an important factor of photosynthesis, we may consider heterogeneity in hydration of different thallus parts as a possible reason for the non-linearity of Φ_{PS2}/Φ_{CO2} in a lichen thallus. Heterogeneity of PS 2 might be another reason of the non-linearity. Most lichen species start to decrease P_N at suboptimal water saturation deficit (WSD) higher than 60 % (Hájek *et al.* 2001, Lange 2002). More pronounced dehydration stress of a lichen thallus leads to strong inhibition of photosynthetic processes. On the other hand, some authors (*e.g.* Lange 2003b) report inhibition of photosynthetic processes in lichens oversaturated by water due to reduction of CO_2 supply caused by increased resistance of diffusion of CO_2 molecules into intrathalline spaces. Therefore, WSD of 0 - 20 % may also have inhibitory effect on lichen photosynthesis.

We found that osmotic stress led to an increase in non-photochemical quenching, its q_{T+I} component in particular. Since under such conditions, less absorbed photon energy is utilised in biochemical processes of photosynthesis, higher share of energy is dissipated in non-photochemical pathway, *e.g.* cyclic electron transport around PS 2, Mehler reaction, creation of Chl-Chl dimers, conversion of violaxanthin to zeaxanthin, aggregation of LHC 2, and inactivation of the OEC, as reviewed by Pospíšil (1997). Hence in osmotically-dehydrated cells of algal symbiont (*T. erici*), q_E is a major component of NPQ.

In conclusion, the study showed that, contrastingly to control, osmotically-stressed *Trebouxia erici* exhibited non-linear relation of Φ_{PS2} to OER. The non-linearity should be taken into account in ecophysiological studies made on lichens using exclusively Chl fluorescence technique in the field. In some cases, partial dehydration in particular, P_N can not be directly derived from Φ_{PS2} due to unpredictable Φ_{PS2} to P_N relation. Thus, for precise evaluation of photosynthetic processes in partially dehydrated lichens, simultaneous fluorometric (Φ_{PS2}) and gazometric (P_N) measurement is a necessity.

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