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REGULATIVE DOWN REGULATION OF PHOTOSYSTEM II BY WATER STRESS: A CHLOROPHYLL FLUORESCENCE STUDY ON LICHENS

Regulative Abschaltung des Photosystem II durch Wasserstreß: Eine Chlorophyll-Fluoreszenz-Studie an Flechten

by

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Key words: Lichens, photosynthesis, chlorophyll fluoresence, water stress, desiccation, *Peltigera aphthosa*.

Schlagwörter: Flechten, Photosynthese, Chlorophyll-Fluoreszenz, Wasserstreß, Austrocknung, *Peltigera aphthosa*.

Abbreviations: PS-photosystem, RH-relative humidity, DBMIB-dibromothymoquinone

Summary: The lichen *Peltigera aphthosa* was studied to determine the response of fluorescence yield and O_2-production to decreasing water potentials. Dehydration took place either by atmospheric desiccation or by osmotic stress produced by incubation of the thalli in NaCl or sucrose solutions. For dehydration and osmotic treatment similar reversible inactivation patterns were obtained. A gradual decline of O_2-production, the parameter $\triangle F/F_m'$ (proportional to photosynthetic electron transport rate) and the fluorescence parameter F_v/F_m (potential electron transfer in photosystem (PS) II) was observed for decreasing water potentials in all cases. At relatively mild water stress the non-photochemical fluorescence quenching (q_N) considerably increased, while the photochemical quenching (q_P) and the Fo level remained almost unchanged. Below about -12 MPa, the variable part of chlorophyll fluorescence became very small and reliable quench parameters could not be determined any more. At this „late" stage the onset of a F_o-quench could be observed. Our data indicate that the

inactivation of photosynthesis during drying of green photobionts takes place in two phases. Initially the relatively high q_P values indicate the absence of overreduction effects on the acceptor side of PS II. The observed fluorescence changes may therefore be due to donor side limitation of electron transport or an unknown quench effect within PS II. At more severe stress conditions (= second phase) the energy transfer in the antennae pigment system probably becomes disturbed.

Zusammenfassung: Die Einwirkung von Wasserstreß auf die Flechte *Peltigera aphthosa* wurde durch Messungen der Chlorophyllfluoreszenz und des Sauerstoffgaswechsels untersucht. Der Wasserentzug wurde entweder durch Abtrocknung oder durch Inkubation in hochkonzentrierten hypertonischen NaCl- bzw. Saccharose-Lösungen induziert. Die Photosynthese wurde durch Abtrocknung und osmotische Behandlung in sehr ähnlicher Weise reversibel abgeschaltet. Mit sinkendem Wasserpotential waren in allen Fällen eine allmähliche Abnahme der Sauerstoffbildungsrate sowie der Fluoreszenzparameter $\Delta F/F_m'$ (proportional zur photosynthetischen Elektronentransportrate) und F_v/F_m (potentieller Elektronentransfer in Photosystem (PS) II) zu beobachten. Bei relativ mildem Wasserstreß erhöhte sich die nicht-photochemische Fluoreszenz-Löschung (q_N) beträchtlich, während die photochemische Löschung (q_P) und die Grundfluoreszenz (F_o) relativ konstant blieben. Ein echtes Absinken des F_o-Wertes setzt erst bei extremem Wasserverlust (unterhalb von ca. -12 MPa) ein. Die variable Fluoreszenz bricht dann zusammen. Die erhaltenen Ergebnisse zeigen eine 2-stufige Inaktivierung von PS II an. In der ersten Phase zeigt der relativ hohe q_P-Wert die Abwesenheit von Überreduktionseffekten auf der PS II Akzeptorseite an. Donorseitige Inaktivierung oder ein unbekannter Löschungseffekt im PS II dürften daher für die beobachteten Effekte verantwortlich sein. Unter starkem Wasserstreß (= zweite Phase) ist zusätzlich wahrscheinlich der Antennenbereich für die Herunterregelung der Photosynthese verantwortlich.

Introduction

Lichens exhibit a remarkable ability to switch their photosynthesis rapidly off and on, when they are dehydrated and rewetted. The mechanistic aspects of this reversible photosynthesis inactivation are not well understood.

The interpretation of fluorescence results is difficult, when working with whole lichen thalli due to optical effects in the upper cortical layer. In addition drying in laboratory air leads to an uneven desiccation within thallus pieces. Thinner border regions desiccate earlier than the central part, a fact that can very easily be observed for the cortex colour changes in *L. pulmonaria* or *P. aphthosa*. These colour changes have recently been shown to be due to the rapid development of intracellular gas holes in the cortex layer (Scheidegger et al. 1995, Honegger et al. 1996). In order to circumvent these difficulties, we have simulated the drying process by exposing the thalli to concentrated solutions of sugar and NaCl as osmotica (Nash III et al. 1990). Our aim was to compare the pure osmotic and concentration effects on chlorophyll fluorescence signals with those of drying in order to gain insight into the reversible inactivation of

photosynthesis during the drying of lichens.

Materials and Methods

Peltigera aphthosa (L.) Willd. was collected on the Inner Bergli, Mathon, Paznauntal, Austria at 0° C. The thalli were kept wet and cold (< 10° C) for 3 days, dried for 4 hours in the laboratory and stored at -25° C until used. After 1-2 years, frozen lichen thalli were rethawed and artificially rewetted by spraying with distilled water. Such samples were stored for at least 15 hours in a refrigerator at about 7° C in darkness, and then for 2 hours at room temperature at about 6 $\mu E \times m^{-2} \times s^{-1}$. This procedure normally leads to a high physiological activity and low zeaxanthin content of the samples (JENSEN et al. 1993).

O_2 -evolution was measured in a LD2 cuvette at 100 $\mu E \times m^{-2} \times s^{-1}$, 22° C and high CO_2 -concentrations (3 drops of 0.5 mol/ L $NaHCO_3$ on a layer of black velvet). For determination of fluorescence parameters a pulse amplitude modulation fluorometer (PAM 101, WALZ) was used. The experiments were performed with samples of 2 cm diameter in the same LD2 chamber. 1.2 s flashes of 5000 $\mu E \times m^{-2} \times s^{-1}$ forced transient closure of all PS II reaction centers and allowed measurement of maximal fluorescence yield F_m. Determination of quenching parameters q_P and q_N was performed according to VAN KOOTEN and SNEL (1990). After two control measurements the samples were dehydrated and measured in a stepwise manner. Dehydration was induced in darkness for 15 minutes either by atmospheric desiccation or by incubation of the thalli in NaCl or sucrose solutions. Excess water or solution on the thallus surface was absorbed with filter paper. Preincubation in 10^{-4} mol/ L dibromothymoquinone (DBMIB) in darkness took 5 minutes. In this case the DBMIB effect was maintained during the following NaCl or sucrose treatments as indicated by low q_P values (not shown). The water content of desiccating samples was determined after each fluorescence measurement. For this purpose the samples were removed from the cuvette and immediately weighed. The actual water content was calculated later according to: 100 (actual weight - dry weight)/ dry weight [%] (LANGE et al. 1970). After the dehydration steps were finished, the samples were rewetted by immersing them in deionized water for 15 minutes and measured again in order to check the reversibility of dehydration effects. The dry weight of the samples was determined after 24 hours of drying over silica gel. All experiments were repeated at least twice with other thalli. There were only minor differences between the investigated thalli. Calibration values of water content versus water potential (data not shown) were obtained by placing thalli for at least 1 day over NaCl solutions adjusted to stepwise decreasing water potential (direction from wet to dry samples). The water potentials in and over the NaCl solutions were calculated according to SLAVIK

1974. The water potentials of sucrose solutions were calculated from freezing point depression data of LEVITT (1956) and SANTARIUS (1992).

Results and discussion

In Fig. 1 the changes of various fluorescence parameters and O_2-production of *P. aphthosa* are shown that took place during dehydration. Changes in water potential were induced either by osmotic stress (NaCl or sucrose solutions) or by atmospheric desiccation. All parameters were changed by drying, and those measured following NaCl and sucrose treatments were very similar. On a water potential basis, however, there was a tendency for drying > NaCl > sucrose in their respective efficiency of inactivation. It should be mentioned that the dehydration experiment were designed on the same short-time basis as the osmotic treatments, i.e. dehydration was not performed by equilibration with a certain relative air humidity (RH), but just by stepwise drying at about 35 % RH (see Materials and Methods). The effects of drying, NaCl and sucrose treatment on *L. pulmonaria* and *H. physodes* were comparable to those obtained on *P. aphthosa* (data not shown). The sensivity series of photosynthesis to water stress was *P. aphthosa* > *H. physodes* > *L. pulmonaria*. All dehydration effects in both hypertonic solutions and under conditions of atmospheric desiccation were reversible after short periods (minutes) of incubation in pure water. This is another example of the extraordinary ability of green algal lichens to tolerate the loss of water to almost complete dehydration and to restore their photosynthetic activity within minutes after rewetting with liquid water or by water vapor uptake (FEIGE and JENSEN 1987, COXSON 1988, LANGE et al. 1989).

At mild water stress conditions a gradual decline of O_2-production, the parameter $\triangle F/F_m'$ [~ photosynthetic electron transport rate, (KRALL and EDWARDS 1992)] and the fluorescence parameter F_v/F_m (efficiency of electron transfer in PS II) was observed. While these fluorescence parameters are considerably diminished, the photochemical quenching parameter q_p stays relatively high, i.e. the photosynthetic electron transport chain remains relatively oxidized. Apparently there is no blocking of the electron transport chain by high proportions of reduced PS II acceptors (= overreduction; cf. FOCK et al. 1992). Simple acceptor side limitation of photosynthesis does not play a crucial role in the inactivation process.

In contrast to q_p, the non-photochemical fluorescence quenching q_N considerably increased in the same range of water potential. The behaviour of quenching parameters is similar to that obtained for *Ramalina maciformis* during hydration by water vapor and uptake by addition of liquid water (LANGE et al. 1989). The q_N increase could indicate an increased transthylakoidal $\triangle$pH due to PS I driven cyclic electron transport (HEBER et al. 1995). FORK and HERBERT (1993) emphasize that cyclic electron flow occurs particularly under stressful

conditions, even at low light intensities. However, q_N also increased during osmotic dehydration in the presence of DBMIB, when plastoquinol reoxidation is inhibited (Fig. 2). These conditions should normally also inhibit cyclic electron flow.

Alternatively, the q_N increase could have been induced by changes in the xanthophyll cycle pigments. The xanthophyll cycle has an influence on the fluorescence yield of lichen thalli (DEMMIG-ADAMS et al. 1990a, b, JENSEN et al. 1993). All our dehydration and osmotic treatments were performed in darkness. However the formation of zeaxanthin and possible photoinhibition effects cannot be completely ruled out. Whether a part of the q_N is caused by formation of zeaxanthin during 7 min of actinic illumination is unknown. After switching off the actinic light, the F_o' values (VAN KOOTEN and SNEL 1990) in the DBMIB experiments were considerably lowered (results not shown). This would fit not only with a possible zeaxanthin formation but also with an enhanced PS I excitation (state transition), enhanced cyclic electron flow and an increased $\triangle$pH (FORK and HERBERT 1993). On the basis of our data it was impossible to distinguish between quenching caused by an increased $\triangle$pH, zeaxanthin formation during illumination, or other direct influences of desiccation on fluorescence yield.

At the initial phase of water stress, the F_o level is only slightly affected. We therefore infer that the majority of fluorescence changes are quenching effects in the vincinity of PS II reaction centers (BUTLER 1978, GIERSCH and KRAUSE 1991) and not changes in the bulk antennae system. In agreement with our data investigations of the fast fluorescence transient (Kautsky curve) of free living green algae and higher plants are interpreted as a limitation on the donor side of PS II due to desiccation (WILTENS et al. 1978, SCHWAB et al. 1989). We accept this interpretation for the first phase of water stress.

At more severe water stress conditions the variable fluorescence (F_v) vanishes completely, the determination of quenching parameters becomes uncertain and a considerable F_o decline was observed (Table 1).

Usually a large decrease in F_o is interpreted as participation of the pigment apparatus in the down regulation of photosynthesis (BUTLER 1978, GIERSCH and KRAUSE 1991) and this interpretation has also been applied to lichen thalli (LANGE et al. 1989, JENSEN and FEIGE 1991). For example, a marked increase in F_o during rehydration from the desiccated state has been reported in *Ramalina maciformis* (LANGE et al. 1989). In the same lichen an impairment of the functional connection between PS II and the chlorophyll b containing light harvesting complex could be observed by means of fluorescence excitation spectra at 77 K (BILGER et al. 1989). Experiments with free living green algae and higher plants, on the other hand, negate the participation of antennae pigment system in the inactivation process during drying (WILTENS et al. 1978, GOVINDJEE et al. 1981, ÖQUIST and FORK 1982, CHEN and HSU 1995).

In any case, the interpretation of the F_o decline is critical. Optical transparency changes in the upper cortex occur on drying (BÜDEL and LANGE 1994, SCHEIDEGGER et al. 1995) and may interfere with the determination of fluorescence yields. Such an effect cannot be completely ruled out and may to be the reason for the fact that dehydration appeared to be more effective than osmotic stress. Moreover, this may explain why the observed F_o decline started earlier during atmospheric dehydration than during submersion in salt or sucrose solutions of comparable water potentials (Fig. 1, Table 1).

Besides the chlorophyll fluorescence measurements at 77 K, two arguments support the view that the observed F_o decline is a real effect. In experiments with high solute concentrations, the optical system is unaffected (controlled by measurement of back-scattering light), and a considerable F_o diminution could be observed in this case either. Secondly the water potentials were much lower in our experiments than in those conducted with higher plants and *Chlorella*. A clear F_o decline could only be found at very low water potentials. Thus, a true F_o decrease and participation of the pigment apparatus in the down regulation of photosynthesis is very likely, but only at advanced water stress. Whether this is really the case not only under atmospheric but also under osmotic water stress conditions, must still be proven by 77 K fluorescence methods.

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Tables:

Table 1: Dependency of F_o and F_v of *P. aphthosa* on water potential (in MPa) as established with sucrose or NaCl solutions or by atmospheric desiccation. F_o values are arbitrary units (Volts).

Treatment: sucrose			Treatment: NaCl			Treatment: drying		
water potential	F_o	F_v	water potential	F_o	F_v	water potential	F_o	F_v
0	0.25	0.63	0	0.27	0.66	0	0.28	0.62
-12	0.22	0.12	-15.27	0.17	0.02	-9.8	0.18	0.19
-21	0.13	0.02	-19.96	0.16	0.01	-16.6	0.13	0.05

Legends to figures:

Figure 1:
O_2-evolution (– –■– –) and fluorescence parameters (F_v/F_m: --▲--, $\Delta F/F_m$': -·-○-·-, F_o: —◇—, q_N: —○—, q_P: —▽—) of *P. aphthosa* dependent on water potential as established by atmospheric desiccation (A) or with NaCl (B) or sucrose (C) solutions.

Figure 2:
Fluorescence parameters (F_o: —◇—, F_v/F_m: --▲--, q_N: —○—) of *P. aphthosa* dependent on water potential as established with sucrose (A) or NaCl (B) solutions in the presence of 10^{-4} mol/L DBMIB. Below -12 MPa, the determination of the quenching factor q_N was uncertain (low F_v value). Therefore, the connection line to the last q_N value below -12 MPa was omitted.