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Inhibition of photosynthetic processes in foliose lichens induced by temperature and osmotic stress

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Abstract

Negative effects of osmotically-induced dehydration of two foliose lichen species, *Lasallia pustulata* and *Umbilicaria hirsuta*, was studied at physiological (22 °C), low (5 °C) and freezing temperature (-10 °C), using chlorophyll (Chl) fluorescence. In both species, exposure to increasing sucrose concentrations led to a pronounced decrease in potential (F_V/F_M), and actual (Φ_2) quantum yields of photochemical processes in photosystem 2. *L. pustulata* was more sensitive to osmotic stress, because comparable osmotic dehydration inhibited F_V/F_M and Φ_2 more than in *U. hirsuta*. Critical concentration of sucrose that fully inhibited photochemical processes of photosynthesis was 2.5 M, which represented water potential (Ψ_w) of -18.8 MPa. Decrease in background Chl fluorescence (F_0) and increase in non-photochemical quenching (qN) revealed two phases of osmotic stress in lichens: phase I with no change (Ψ_w 0 to -6.6 MPa) and phase II (Ψ_w -11.3 to -18.8 MPa) typical by substantial change in Chl fluorescence parameters. Effects of thallus anatomy on species-specific response to osmotic dehydration is discussed and attributed to the results obtained by optical microscopy and Chl fluorescence imaging technique.

Additional key words: photosynthesis, water potential, dehydration, *Lasallia pustulata*, *Umbilicaria hirsuta*, sucrose, chlorophyll fluorescence, thallus anatomy.

Introduction

In lichens, typical poikilohydric organisms, high air temperature is a major environmental stressor causing thallus dehydration resulting in an inhibition of photosynthetic processes. However, lichens are highly adapted to dehydration. They are capable to survive numerous dehydration cycles within a growing season. Physiological mechanisms of this resistance are not fully elucidated and consequences for photosynthetic processes of their symbiotic photobiont (alga/cyanobacteria) are recently intensively studied. In general, dehydration in lichens might be caused by loss of water at high/low temperature, and/or osmotic stress. Negative effects of dehydration on lichen photosynthetic performance has been studied either alone (*e.g.* Jensen *et al.* 1999, Lange *et al.* 2001) or in combination with other interacting factor such as high irradiance (Gauslaa and Solhaug 1999).

Lichens are capable to cope with perturbations of temperature, radiation, and water availability, in particular by repetitive dehydration and consequent rehydration. In lichens, ongoing dehydration leads to gradual loss of photosynthetic activity, which is manifested as a decrease in the efficiency of absorbed energy transfer through photosystem 2 (PS 2) and net CO_2 fixation. Dehydration-induced changes in PS 2 might be clearly demonstrated by a decrease of variable chlorophyll (Chl) fluorescence and changes of other parameters derived from the Chl fluorescence induction curve. Within last two decades, photosynthetic response of lichens to dehydration has been studied on thalli dehydrated either by atmospheric desiccation (*e.g.* Hájek *et al.* 2001) or osmotic dehydration (*e.g.* Chakir and Jensen 1999).

As concern photosynthetic parameters, consequences of dehydration in lichens (for review see Palmqvist 2000) are: *1*) progressive inhibition of photosynthetic and respiration rate during loss of pressure potential in cells, *2*) protection of photosystems by disconnection of LHC

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Abbreviations: F_V/F_M - potential yield of photochemical reactions in PS 2, Φ_2 - quantum yield of photochemical reactions in PS 2, F_0 - background Chl fluorescence, F_M - maximum Chl fluorescence, qN - non-photochemical quenching of Chl fluorescence, Ψ_w - water potential

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from PS 2 (Bilger *et al*. 1989) or redistribution of excitacion energy from PS 2 to PS 1, and *3*) synthesis of compounds such as sugars and sugar alcohols (Farrar 1988) that can stabilise proteins during dehydration. In addition, several antioxidants (*e.g*. glutathione) are reported to have a protective role during dehydration stress in lichens (Kranner *et al*. 2003). Low and especially freezing temperature leads to direct effects on phospholipidic bilayers, proteins and other macromolecules forming thylakoids (Smallwood and Bowles 2002). Another effect of freezing temperature is a withdrawal of liquid water from cells that induces increased concentration of ions which leads to dehydration of membranes and macromolecules of thylakoids. Freezing temperature also brings a mechanical stress to cells and their organelles due to ice crystal formation. Some lichens, however, have effective protective mechanisms against low temperature effects, such as synthesis of sugars (Montiel 2000) and sugar alcohols (Fontaniella *et al*. 2000).

The aim of our study was to find species-specific responses of two foliose lichens to dehydration caused by osmotic stress (mimicking atmospheric dehydration) combined with the effect of low thallus temperature. These factors act simultaneously during winter in the field. We designed our experimental set up in such a way that they could be studied separately (osmotically-induced dehydration and temperature). This design allowed to analyse dehydration and temperature effects alone and in a combination. The aim of our study was also to quantify osmotically-induced changes in anatomy of thallus, photobiont layer in particular, that are responsible for inhibition of photosynthetic processes in two foliose lichen species.

Materials and methods

Lichen collection, exposure to osmotic stress: Thalli of foliose lichen species, *Lasallia pustulata* (L.) Mérat, and *Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm., were collected in the field in a vicinity of the Ketkovice village, 30 km W of Brno, Czech Republic. After the collection, thalli were transferred to a laboratory, where they were stored under 5 °C at dim light (10 μmol m^{-2} s^{-1}). Twenty four hours before experiments, the thalli were rehydrated between two layers of filter paper supplied with demineralized water. Then thalli were exposed to different levels of osmotic stress (0.2, 1.0, 1.5, 2.0, 2.5 M sucrose) for 48 h. Temperature was the interacting factor. It was set at 22, 5, and -10 °C, respectively, and kept constant throughout the exposure. The sucrose concentrations applied to lichens were chosen according to Jensen *et al*. (1999). We expected very low effect of the smallest concentration (0.2 M sucrose) and a high effect of the highest concentration used (2.5 M sucrose). Water potential (Ψ_w), corresponding to the concentration of sucrose, was calculated by the method of Slavík (1974). The concentration of 2.5 M sucrose is an equivalent to Ψ_w of -18.8 MPa, which represents high water stress even in poikilohydric organisms. The value of about -15 MPa is typical for highly dehydrated but still physiologically active lichen thalli (Jensen *et al*. 1999, Barták and Gloser 2004).

Thallus anatomy: *L. pustulata* and *U. hirsuta* thalli were exposed to 2.5 M sucrose at 22 °C for 48 h. Control thalli of both lichen species were hydrated by demineralized water at the same temperature for 48 h. Thallus segments of 5 × 3 mm in size were taken from 3 different thallus zones: *1*) the central zone near to the umbilicus, *2*) the intermediate zone between the umbilicus and the margin and *3*) marginal zone. The segments were taken from thalli for control and osmotic treatment (2.5 M sucrose). Nonfixed thallus segments were sectioned (10 μm) in *Tissue-Tec O.C.T.*® compound (*Electron Microscopy Sciences*, Tokyo, Japan) using the freeze cryostat (*American Optical Corporation*, Southbridge, Massachusetts, USA). Sections were stretched on glass slides previously treated with chrome alum-gelatine and air-dried. Adhered sections were then rehydrated and mounted in the same liquid with which thalli were pretreated (*i.e*. either 2.5 M sucrose or demineralized water). Images of sections with a special respect of the photobiont layer were accomplished by means of the light microscope (*Olympus BX-51*, Tokyo, Japan) equipped with the digital camera (*Camedia 5050Z, Olympus*). Image analysis, *i.e*. evaluation of the projected area of symbiotic algal cells (*Trebouxia* sp.) as well as the thickness of the thallus were processed by means of the software *Lucia G* (*LIM*, Praha, Czech Republic).

Chl fluorescence measurements: Chl fluorescence parameters (F_V/F_M, F_0, Φ_2, qN) were measured by a *PAM 2000* (*Walz*, Effeltrich, Germany) fluorometer after 24 and 48 h exposure to osmotic stress using a saturation pulse method with quenching analysis. To determine background Chl fluorescence (F_0), a beam of weak irradiance (0.5 μmol m^{-2} s^{-1}) was applied on dark-adapted (10 min) lichen thalli. Then, a pulse of irradiance saturating photosynthesis (5 000 μmol m^{-2} s^{-1} for 0.8 s) was applied in order to induce maximum Chl fluorescence (F_M) and calculate F_V/F_M as $(F_M - F_0)/F_M$. Quantum yield of photochemical reactions in PS 2 (Φ_2) was calculated as $(F'_M - F_S)/F'_M$, where F'_M denotes maximum Chl fluorescence induced by a saturation pulse on radiation-adapted (40 μmol m^{-2} s^{-1} for for 5 min) lichen thalli. At the end of irradiance adaptation interval, Chl fluorescence reached steady-state level (Fs), which was recorded for Φ_2 calculations. Non-photochemical quenching of Chl fluorescence (qN) was calculated according to Schreiber *et al*. (1986) as $(F_M - F'_M)/F_M$, where F_M represents maximum Chl fluorescence measured before addition of sucrose and F'_M is actual level of maximum Chl fluorescence on radiation-adapted thallus

exposed to sucrose. During measurements, the surface temperature of lichen thalli was maintained constant, *i.e.* the same as for individual temperature treatments.

Spatio-temporal distribution of negative effects of osmoticum (2.5 M sucrose) on primary processes of photosynthesis over a lichen thallus was monitored by Chl fluorescence imaging. Chl fluorescence was measured by a PC-linked portable fluorometer *FluorCam HFC-010* (*Photon Systems Instruments*, Brno, Czech Republic) equipped with a CCD camera and image analysis software (*FluorCam 6.0*). The technique provided high resolution false-colour images of Chl fluorescence distribution over the thalli of measured lichens. In the exposure chamber of the instrument, lichen thalli were dark adapted (10 min) at room temperature and then exposed to the experimental protocol (see below) in order to take an image of F_V/F_M and Φ_2 distribution over a thallus. The measurements started with F_0 determination in dark-adapted thalli followed by a maximum Chl fluorescence (F_M) determination after the application of a pulse of irradiance of 1100 μmol m^{-2} s^{-1}. Then, after 10 s of dark, irradiance of 65 μmol m^{-2} s^{-1} was applied for 60 s in order to induce Chl fluorescence reaching steady-state (F_S) at the end of the time interval. Then, another pulse of saturating irradiance was applied in order to reach the maximum of Chl fluorescence in radiation-adapted lichen samples (F_M'). Quantum yield of photochemical reactions in PS 2 (Φ_2) was then calculated as (F_M' - F_S/F_M'). The changes of Chl fluorescence during the whole above-described measuring protocol were sensitively monitored by a fluorometric CCD camera with the time step of 50 ms. Thus, for each pixel of taken images, the kinetics of Chl fluorescence was recorded and basic Chl fluorescence parameters (F_V/F_M, Φ_2) were computed. The images were taken for each lichen thallus before and several times (30 min interval) after exposure of the thalli to 2.5 M sucrose in order to visualise gradual osmotically-induced decline of the two Chl fluorescece parameters. The images were then analysed and thalli parts exhibiting minimum and maximum values of the above Chl fluorescence parameters were chosen for detailed analyses of the effects of sucrose and time of exposure.

Results

Distribution and size of *Trebouxia* cells in lichen thallus: The *L. pustulata* thallus was typical by numerous hemispheric parts called pustulas. Therefore, it had cells of *Trebouxia* photobiont arranged very irregularly in the algal layer. The greatest number of photobiont cells was concentrated into algal layer of pustulas, whereas markedly lower number of these cells was located in the parts of the thallus between pustulas. Furthermore, gradual lowering of algal cell number was observed in radial direction either in pustulas or between them (Fig. 1*A,B,C*).

Flat thallus of *U. hirsuta* revealed more constant thickness of algal layer than that of *L. pustulata*. *U. hirsuta* thallus thickness was of about 325 μm in central part and decreased to 230 μm at the thallus margin. Similar center-to-margin gradient was observed in *L. pustulata*, when considering thallus thickness measured between pustulas. Quantity of algal cells on sections was much greater in *U. hirsuta* than in *L. pustulata* (Fig. 1*A,G*), nevertheless, gradual reduction of their number in the direction from central part to the thallus margin was also observed (Fig. 1*G,H,I*).

Projected area of algal cells on the sections of thallus segments was used as the parameter for estimation of the *Trebouxia* cell size. It was measured in both lichen species after either rehydration of cryosections in demineralized water (control conditions) or osmotic dehydration by 2.5 M sucrose. In control (demineralized water), the areas of the photobiont cells in *U. hirsuta* thallus were slightly greater in comparison with that of *L. pustulata* thallus (Fig. 2*A,B*). Projected area of algal cells were the smallest in the central segments of both studied lichens in comparison with the intermediate and marginal segments of the thallus.

Osmotic dehydration with 2.5 M sucrose caused protoplast contraction in *Trebouxia* cells in both species (Fig. 1*D,E,F,J,K,L*). Photobiont size decrease was more prominent on sections of the *L. pustulata* thallus (Fig. 2*A,B*), where the area of the photobiont cells decreased in central, intermediate and marginal segment to 70, 57 and 52 % of the cell area of control, respectively. Osmotically induced diminutions of the photobiont cells area on the sections of *U. hirsuta* thallus from the central part to the thallus margin were to 88, 64 and 72 % of control.

Thallus thickness was not significantly influenced by osmotic dehydration. Nevertheless, *L. pustulata* displayed noticeable thallus undulation, manifested by a marked thallus contraction that led to the decrease of projected thallus area (Fig. 7). Thallus layers on cryosections were better preserved in those cases, when the thalli were exposed to the osmotic treatment before cryosectioning.

Effects of sucrose and temperature on Chl fluorescence: Sucrose-induced osmotic stress led to changes in all studied Chl fluorescence parameters. Time courses of background Chl fluorescence (F_0) taken for lichens treated with sucrose showed different response to its low and high concentrations. While sucrose concetration from 0.2 to 1.5 M generally caused either no change or an increase in F_0, higher sucrose concentration (2.0 and 2.5 M) caused dramatic decrease in F_0. For all experimental temperatures, lichens treated with 2.0 and 2.5 M sucrose for 48 h showed F_0 decrease to 20 - 40 % of pre-treatment value. The decrease was more pronounced in *L. pustulata* than in *U. hirsuta* (Fig. 5).

The ratio of variable to maximum fluorescence (F_V/F_M) decreased in both studied lichen species in response to increasing concentration of sucrose and time

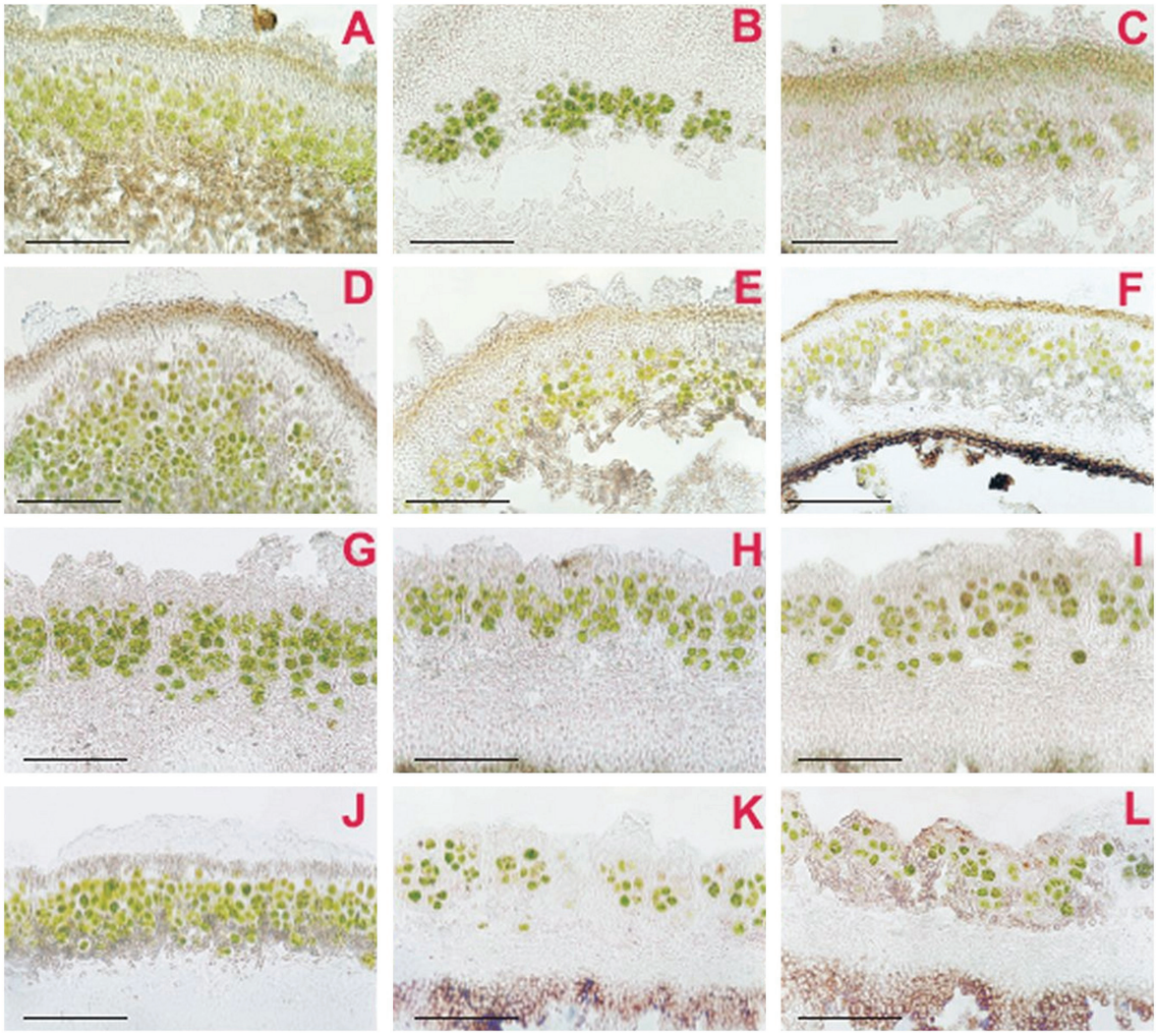

Fig. 1. Cryosections of the *Lasallia pustulata* (*A-F*) and *Umbilicaria hirsuta* (*G-L*) thallus in different distances from the thallus center. *Lasallia pustulata*: central zone (*A*,*D*), intermediate zone (*B*,*E*) and marginal zone (*C*,*F*). Sections of the top row (*A*,*B*,*C*) were taken from thalli treated with demineralized water, the second row (*D*,*E*,*F*) with 2.5 M sucrose. *Umbilicaria hirsuta*: central zone (*G*,*J*), intermediate zone (*H*,*K*) and marginal zone (*I*,*L*). Sections of the third row (*G*,*H*,*I*) were taken from thalli treated with demineralized water, bottom row (*J*,*K*,*L*) with 2.5 M sucrose. *Bar* represents 100 µm.

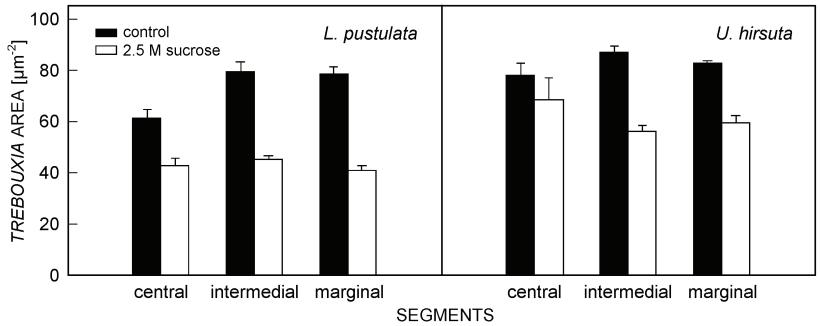

Fig. 2. Comparison of *Trebouxia* sp. projected cell area measured on the cryosections of the *Lasallia pustulata* and *Umbilicaria hirsuta* thalli treated with 2.5 M sucrose and demineralized water (as a control) for 48 h. *Error bars* represent ± SD.

of exposure. Higher concentrations caused more pronounced decrease in F_V/F_M (Fig. 3). Full loss of functioning primary photochemistry in PS 2 (zero F_V/F_M) was recorded for thalli treated with 2.5 M sucrose for 24 h at 22 °C. This was more apparent in *L. pustulata* than *U. hirsuta*. After 48 h exposure, the loss of F_V/F_M was found also in lichen thalli treated with 2.0 M sucrose. At 22 °C, lowest concentration of sucrose (0.2 M for 48 h) corresponding to Ψ_w of -0.5 MPa caused reduction in F_V/F_M to about 40 and 80 % of pre-exposure values in *U. hirsuta* and *L. pustulata*, respectively. Low temperature during exposure had positive effects on F_V/F_M values. The decrease in F_V/F_M caused by sucrose was apparent at 5 °C and -10 °C, however, the absolute values recorded for particular concentrations and exposure were higher than those recorded at 22 °C. While at 22 °C, F_V/F_M decreased in all sucrose concentrations, at 5 °C, no significant decrease in F_V/F_M was found for 0.2 and 1.0 M sucrose concentration. At sub-zero temperature (-10 °C), moreover, no decrease in F_V/F_M was recorded even in lichen thalli treated by 1.5 M sucrose solution. Our results pointed out interspecific difference of F_V/F_M in response to osmotic stress. For all concentrations and temperatures used, *L. pustulata* showed somewhat lower F_V/F_M values than *U. hirsuta*, which might indicate higher sensitivity of primary photochemical processes of *L. pustulata* to osmotic stress.

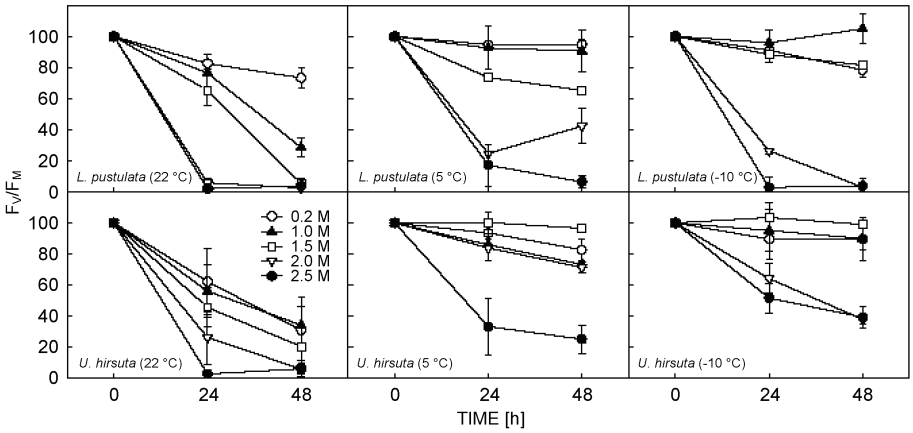

Fig. 3. Time courses of F_V/F_M decrease in *Lasallia pustulata* and *Umbilicaria hirsuta* as dependent on sucrose concentration and thallus temperature. Means of three replicates ± SD.

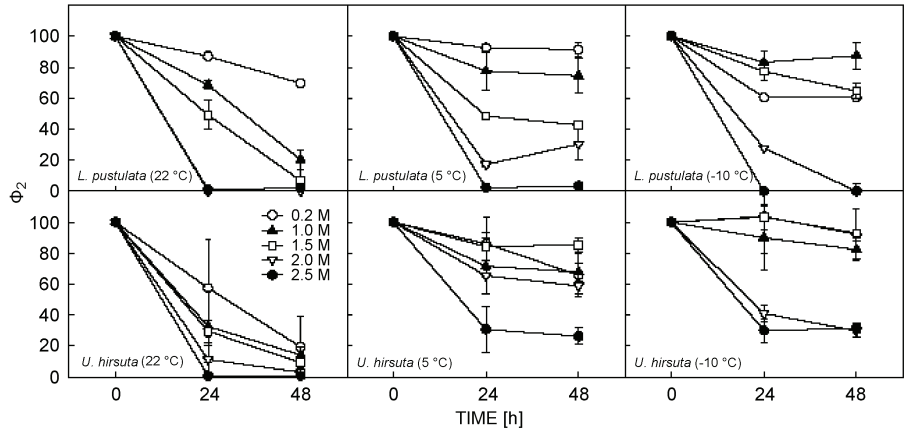

Fig. 4. Time courses of Φ_2 decrease in *Lasallia pustulata* and *Umbilicaria hirsuta* as dependent on sucrose concentration and thallus temperature. Means of three replicates ± SD.

Generally, quantum yield of electron transport through PS 2 (Φ_2) decreased with increasing sucrose concentration and exposure under different temperatures (Fig. 4). Response of Φ_2 was similar to the responses of F_V/F_M. In both species treated at 22 °C, 2.5 M sucrose caused full inhibition of photochemical photosynthetic processes after 24 h exposure. The same concentration, when applied at 5 °C and -10 °C, respectively, led to full inhibition of Φ_2 in *L. pustulata*, while in *U. hirsuta* Φ_2 decreased to about 25 % of the pre-treatment value. In general, negative effect of osmotic stress on Φ_2 was less apparent at low (5 °C) and freezing (-10 °C) temperatures. For sucrose concentrations of 0.2 and 1.0 M, much higher decrease in Φ_2 was found at 22 °C (by 20 - 80 %

in both species) than at 5 °C (10 - 25 % in both species). *L. pustulata* showed lower resistance to osmotic stress than *U. hirsuta* because it exhibited lower Φ_2 values for comparable sucrose concentrations and exposure, especially under low (5 °C) and freezing (-10 °C) temperatures.

Osmotic stress led to an increase in non-photochemical quenching (qN) in both studied species (Fig. 6). The increase was most apparent for the highest concentration of osmoticum (2.5 M sucrose). Maximum qN value was recorded for 2.5 M-treated thalli and reached value of about 1 (theoretical maximum) after 48-h exposure. Compared to control, lowest concentration of sucrose (0.2 M) led to an increase of qN of about 2 to 3 folds after 24 and 48 h exposure, respectively. In both studied species, values of qN increased with increasing concentration of sucrose and time of exposure. The latter was true mainly for optimum temperature (22 °C); under 5 and -10 °C, the qN dependence on time of exposure was less apparent. Our data, however, suggested inter-specific differences in qN under small to moderate osmotic stress. After 48 h exposure of thalli to 0.2 - 1.0 M sucrose, *L. pustulata* showed lower increase in qN than *U. hirsuta*.

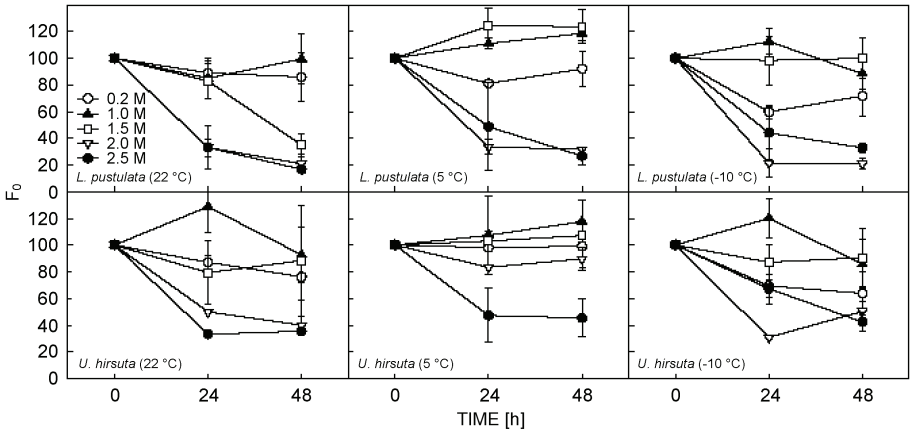

Fig. 5. Time courses of F_0 decrease in *Lasallia pustulata* and *Umbilicaria hirsuta* as dependent on sucrose concentration and thallus temperature. Means of three independent replicates ± SD.

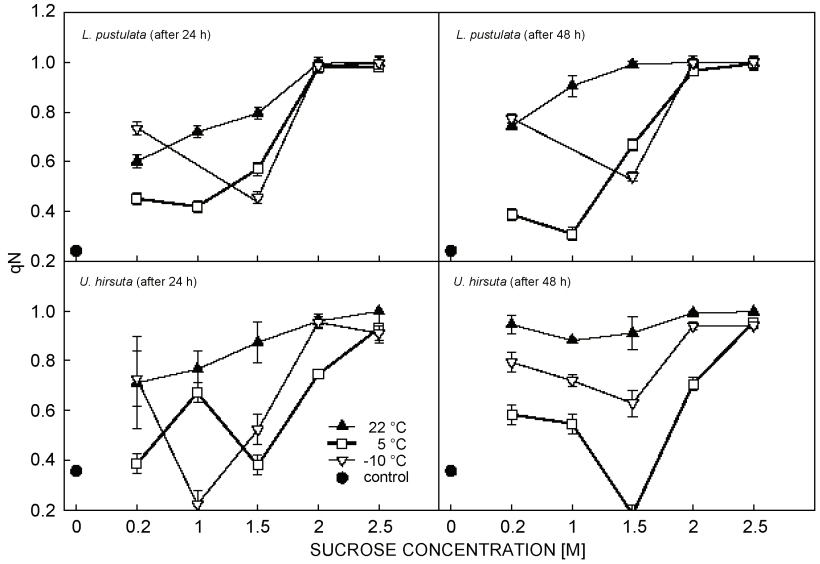

Fig. 6. Relation of non-photochemical quenching (qN) to sucrose concentration. Thalli of *Lasalllia pustulata* and *Umbilicaria hirsuta* were exposed to sucrose at 22, 5, and -10 °C. Pre-exposure value of qN is indicated by full circles. Means of three replicates ± SD.

Chl fluorescence imaging: *L. pustulata* and *U. hirsuta* showed a species-specific heterogeneity in spatio-temporal distribution of F_V/F_M and Φ_2 (Fig. 7) over thalli. Before exposure to 2.5 M sucrose, F_V/F_M and Φ_2 reached mean value of 0.718 and 0.520 in *L. pustulata*, respectively. The two Chl fluorescence parameters were more or less evenly distributed over the thalli. However, some apparently confined local F_V/F_M maxima were

apparent in *L. pustulata*. Location of the maxima corresponded to pustulas (verrucoid outgrowths). In thalli of *U. hirsuta*, few areas exhibiting F_V/F_M higher than mean were located in thallus centre, close to umbilicus. Exposure to sucrose led to the inhibition of photosynthetic activity. First signs of the inhibition were manifested after 30 min exposure. Strong decrease of F_V/F_M and Φ_2 was found in marginal parts of *L. pustulata* thallus. In *U. hirsuta*, the F_V/F_M and Φ_2 decrease was irregularly distributed to marginal and sub-marginal thalli parts. In *L. pustulata*, 30 min exposure led to strong contraction of thallus resulting in a decreased thallus area. The osmotically-induced thallus contractions was much more apparent in *L. pustulata* than in *U. hirsuta*. Inhibition of primary photosynthetic processes and contemporary reduction of thallus size were more pronounced with time of exposure. This resulted in a strong and evenly distributed reduction in F_V/F_M (0.2) and Φ_2 (0.2) in *L. pustulata* after 3 - 4 h. Similar reduction was seen in *U. hirsuta*, however, some close-to-centre thalli parts exhibited somewhat higher F_V/F_M values than marginal and sub-marginal parts. In this species, Φ_2 was homogeneously reduced over the thallus area reaching the value of 0.2. Time courses of F_V/F_M and Φ_2 decreased

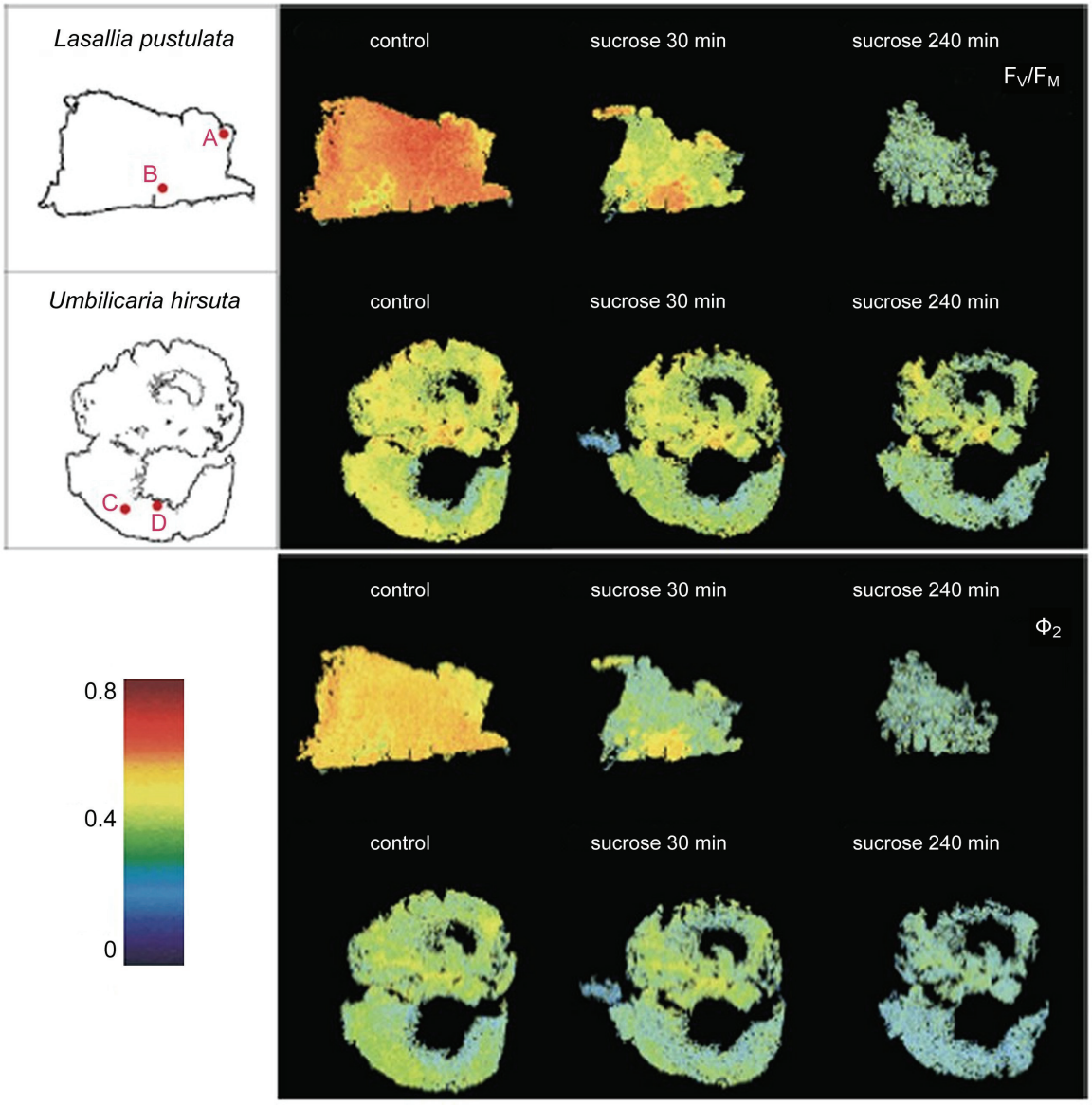

Fig. 7. False colour images of F_V/F_M (*upper panel*) and Φ_2 (*lower panel*) distribution over thallus as dependent on osmotic stress: control - thallus in demineralized water, sucrose - the same thallus exposed to 2.5 M sucrose for the indicated time. On left-sided drawings points (A, B, C, D) exhibited contrasting photosynthetic activity: A - thallus margin, low activity, B - thallus center, high activity, C - intermediate thallus part, high activity, D - intermediate thallus part, low activity. For these points, analysis of F_V/F_M and Φ_2 time courses of osmotically-induced decline were done (see Fig. 8). In the individual panel: *Lasallia pustulata* (*upper row*), *Umbilicaria hirsuta* (*lower row*).

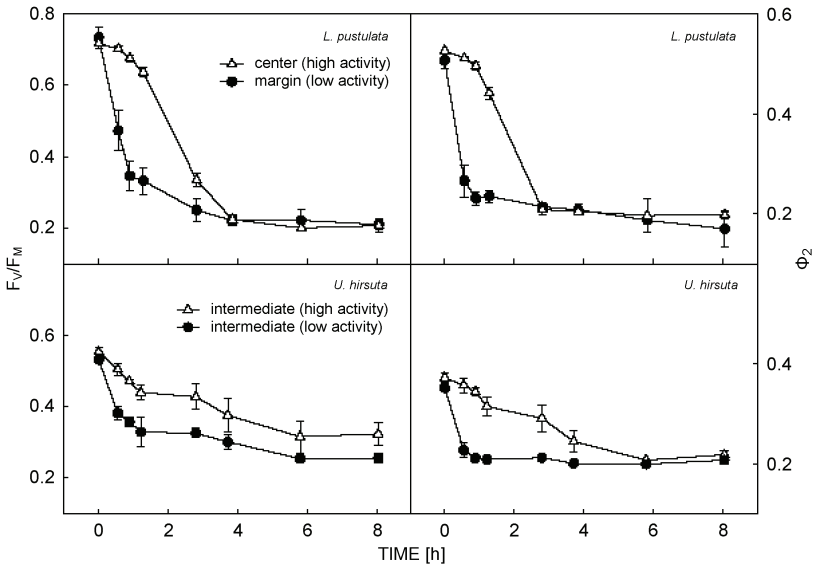

Fig. 8. Time courses of F_V/F_M and Φ_2 in osmotically-treated (2.5 M sucrose) thalli of *Lasallia pustulata* and *Umbilicaria hirsuta*. The data were taken for the points on the thalli defined in Fig. 7 that exhibited high (*B,C*) and low (*A,D*) photosynthetic activity. Means of three replicates ± SD.

upon time of exposure (Fig. 8). The curves pointed out species-specific response of the parameters to to gradual infiltration of sucrose into a thallus. While in marginal thallus parts, fast drop in F_V/F_M and Φ_2 was seen in both species, in central parts, the species exhibited different responses. In *L. pustulata*, high F_V/F_M and Φ2 retained for 1 h and then dropped to minimum values comparable to those recorded in thallus marginal parts. In *U. hirsuta*, contrastingly, F_V/F_M and Φ_2 exhibited gradual loss with time of exposure in close-to-centre thallus parts. Minimum Φ_2 comparable to that found in marginal part was reached after 6 h. At the same time, slightly but significantly higher F_V/F_M values were recorded in close-to-centre thallus than in marginal parts

Discussion

Thallus anatomy under osmotic stress: *Trebouxia* sp. cells in osmotically treated thalli of *L. pustulata* and *U. hirsuta* were densely packed similarly as was reported by De los Ríos *et al*. (1999) for desiccated *Lasallia hispanica*. The same authors showed that the layer of *Trebouxia* sp. cells appeared closer (compared to the hydrated thallus) to the upper cortex layer as a result of desiccation in *Parmelia omphalodes*. In our experiments, this phenomenon was not distinctly observed in either *L. pustulata* or *U. hirsuta*, as changes of the vertical thallus thickness were not significant after sucrose treatment. We found, however, interspecific differences of photobiont cell sizes in response to osmotic stress. The projected area of photobiont cells on thallus sections of *L. pustulata* in control conditions (demineralized water) increased from the thallus centre to the marginal parts. The impact of the osmotic dehydration (Fig. 2) to the *Trebouxia* sp. cells was greatest in marginal zones, where cell area was about 52 % of control cells, while in the central zone, the cell size was reduced only to 70 % (in comparison to controls). Changes of photobiont size on the sections of the thallus of *U. hirsuta* after sucrose treatment were not so prominent (projected area of photobiont cells averaged 72 % of control in marginal zone and 88 % in the central zone).

Tissue-Tec O.C.T.® compound was used as a supporting medium for cryosectioning in both treatments but structures of thallus layers were better preserved on cryosections from thalli exposed before sampling and sectioning to sucrose. These results are in agreement with the fact that osmotic dehydration with sucrose is often used for cryopreservation of plant tissues (Benson 1994, Harding and Benson 1995) or that sugars can be synthesised by lichens as a result of their exposure to low temperatures (Montiel 2000). Photobiont cells on sections were capable to rehydrate very quickly after mounting to glycerol solution. Therefore, thallus section had to be mounted into the same medium as was used in pre-treatment (*i.e.* demineralized water and 2.5 M sucrose), which caused different optical properties of microscopic preparations.

Chlorophyll fluorescence under osmotic stress: Decreased Φ_2 and F_V/F_M values recorded after 24 and 48 h exposure to sucrose indicated strong inhibition of photochemical processes of photosynthesis caused by osmotically-induced dehydration. The decrease is typical for desiccating poikilohydric plants and lichens and has

been reported by numerous authors (*e.g.* Deltoro *et al.* 1998b, Hájek *et al.* 2001). Our data suggest that the first detectable inhibition of photochemical processes was caused by 0.2 M sucrose which corresponds to Ψ_w of -0.5 MPa. Critical Ψ_w (calculated from critical osmotic concentrations), under which photochemical processes of photosynthesis were fully inhibited (Φ_2 and F_V/F_M close to zero); reached -11.3 to -18.8 MPa. This range is comparable to experimental evidence reported by Chakir and Jensen (1999) and Jensen *et al.* (1999). Their Chl fluorescence data measured on three osmotically-affected foliose lichen species (*Hypogymnia physodes*, *Lobaria pulmonaria*, *Peltigera aphtosa*) suggest critical Ψ_w of about -20 MPa. In atmospherically-desiccated lichens, critical Ψ_w for photochemical processes of photosynthesis was -15 MPa (Jensen *et al.* 1999) for the same species. Somewhat lower and more species-specific Ψ_w was found as critical for photosynthetic processes in several atmospherically-desiccated Antarctic foliose and fruticose species (Barták and Gloser 2004) suggesting interspecific differences in lichen resistance to desiccation. We may, therefore, conclude that critical Ψ_w for lichen photosynthesis lies in the range of -15 to -25 MPa, but interspecific differences might exist. These differences might be attributed to a thallus anatomy of individual lichen species, thickness of cortex layer, its density and physical properties. Actual content of osmotically-active compounds in a lichen thallus, polyols in particular, might be considered as another reason for interspecific differences in lichen response to dehydration, since a wide ranges of ribitol and mannitol concentrations, have been reported by Dahlman *et al.* (2004) in green algal lichens: 0.8 - 2.6 mg(ribitol) g^{-1}(d.m.), and 0.6 - 6.2 mg(mannitol) g^{-1}(d.m.), respectively.

In our experiment, non-photochemical quenching (qN) increased with pronounced osmotic stress exhibiting full inhibition of photosynthetic processes (qN = 1, Φ_2 = 0) at Ψ_w below -18.8 MPa. Similar results are reported for non-photochemical quenching in dehydrating lichens (*e.g.* Hájek *et al.* 2001), mosses (Deltoro *et al.* 1998a) and liverworths (Deltoro *et al.* 1998b). In these studies, dramatic increase in non-photochemical quenching was found when relative water content of the poikilohydric organisms decreased below 10 %. Increase in non-photochemical quenching is considered as a measure of protection against damage to PS 2 (Weiss and Berry 1987) that downregulates efficiency of electron transport through PS 2. Our data indicate that such protection is activated in *L. pustulata* and *U. hirsuta* when Ψ_w decreases below -0.5 MPa. Such effective involvement of non-photochemical pathway of energy dissipation in poikilohydric organisms might be attributed to dehydration-induced zeaxanthin formation (Casper *et al.* 1993, Calatayud *et al.* 1997), thermal dissipation (Hamerlynck *et al.* 2000), and production of antioxidants (Augusti *et al.* 2001). These mechanisms are involved in the protection of PS 2 and thylakoid membrane components against reactive oxygen species that are formed by numerous stresses imposed to lichens, such as dehydration (Kranner *et al.* 2003) or high irradiance (Barták *et al.* 2004).

Effects on background Chl fluorescence: Negative effects of desiccation on decreased functioning and effectivity of PS 2 in algal lichens has been frequently documented (*e.g.* Calatayud *et al.* 1997, Hájek *et al.* 2001). In our study, F_0 decreased with strong osmotic stress and linear relationship was found between qN and qF_0 (data not shown). F_0 decreases with ongoing osmotic dehydration and relation of non-photochemical quenching to qF_0 might be indicative for osmotically-induced antennae-type quenching (Jensen *et al.* 1999). This protective mechanism increases stability of PS 2 and maintains its function even under chronic and/or episodic stresses. If osmotic dehydration is applied instead of atmospheric, the other possible causes of F_0 decrease, such as *e.g.* change of optical properties of the upper cortex during atmospheric desiccation (Scheidegger and Schroeter 1995), might be ruled out because a thallus is immersed in osmolyticum during dehydration. Our data on F_0, when compared to similar study in three foliose lichen species (Jensen *et al.* 1999), brought supporting information about osmotic effects on F_0. Our data suggest decrease in F_0 at Ψ_w lower than -11.3 MPa, similarly to Jensen *et al.* (1999) who reported significant decrease in F_0 for sucrose concentrations corresponding to a Ψ_w range of -7.3 to -14.4 MPa. Under lower sucrose concentrations used in our experiment, (0.2 to 1.5 M, *i.e.* Ψ_w of -0.5 to -6.6 MPa), either no change or an increase in F_0 was recorded, more apparently at low temperature (Fig. 5), comparably to Chakir and Jensen (1999) who reported no decrease in F_0 within a Ψ_w interval of 0 to -10 MPa for *Lobaria pulmonaria*.

Low temperature effects: Our experiment investigated an interaction of osmotically-induced desiccation and low temperature in regulation of primary photochemical processes. Under freezing temperature, the negative effects of the same sucrose concentrations on Φ_2 and F_V/F_M were less pronounced that at 22 °C. Tolerance of lichens to freezing temperatures might be generally attributed to the effects of sugar alcohols (Fontaniella *et al.* 2000), antifreezing and ice nucleation promoting proteins (Kieft and Ruscetti 1992) which are expressed at subzero temperature (reviewed *e.g.* by Elster and Benson 2004). Antifreezing activity related to these proteins is reported for numerous lichen species (Doucet *et al.* 2000). Previous study (Hájek *et al.* 2001) showed that exposure of hydrated lichen thallus to subzero temperature led to about 60 % reduction of Φ_2 in *Cetraria islandica* compared to maximum Φ_2 at 22 °C. Our experiment with *L. pustulata* and *U. hirsuta*, on the contrary, showed rather higher F_V/F_M and Φ_2 values at 5 °C and -10 °C than at 22 °C. This effect might be attributed to a positive role of experimentally added sucrose in cells of photobiont at the low temperatures. Added sucrose, similarly as reported for sugar alcohols, had positive effect on physiological status and

performance of photobiont cells at freezing temperature, stability of membranes and their components in particular. In this way, photosynthetic activity under subzero temperature was improved and freezing of thallus was postponed by sucrose addition (data not shown). However, our data do not allow to distingush whether the thalli were completly frozen at -10 °C at low sucrose concentration (0.2 and 1.0 M, respectively) and what the proportion between extra- and intra-cellular freezing was within the thalli.

F_0 is generally considered as temperature-independent within physiological temperature range of -10 to 30 °C, however, slightly increasing F_0 is reported with temperature decrease within the above mentined temperature interval (Pospíšil *et al*. 1998). Our data showing slightly higher F_0 values at 5 °C, and -10 °C than at 22 °C for all experimental osmotic treatments are consistent with general findings about dependence of F_0 on temperature. Since F_0 is attributed mainly to Chl fluorescence coming from LHC, we may interpret our data as low temperature-induced detachment of LHC from core of PS 2 and thus increased emission of Chl fluorescence from detached LHCs. This mechanism might be considered as protective causing reduction of excitation energy in PS 2.

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