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Responses of Plastid Pigments to Desiccation and Rehydration in the Desert Lichen *Ramalina maciformis*

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Abstract

Plastid pigments were analysed in thalli of the desert lichen *Ramalina maciformis* (Delile) Bory during a desiccation period of 30 d, and during rehydration following short-term (6 d) and long-term (30 d) desiccation. Desiccation and rehydration were performed in either constant light or constant dark. While further desiccation of freshly collected, air dried thalli did not affect the contents of violaxanthin and zeaxanthin, conversion from zeaxanthin to violaxanthin occurred during rehydration, regardless of whether lichens have been exposed to constant light or dark. In addition, *de novo* synthesis of violaxanthin took place during rehydration of short-term desiccated thalli exposed to constant light. Neither desiccation nor rehydration affected the contents of lutein, neoxanthin, antheraxanthin and β-carotene. Chlorophyll a and b, and α-carotene were slightly increased or decreased, respectively, during rehydration.

Keywords: Plastid pigment, xanthophyll cycle, lichen, desiccation, *Ramalina maciformis*

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1. Introduction

Desert plants are adapted to extreme conditions, including high irradiance, temperature and drought. Under such conditions the formation of free radicals increases (for review see Smirnoff, 1993; Elstner and Osswald, 1994; Bartosz, 1997). Tolerance to oxidative stress requires the capacity to scavenge free radicals and reactive oxygen species, which is performed by antioxidants such as glutathione, tocopherols, β-carotene, ascorbic acid, and related enzymes including superoxide dismutase, peroxidase, catalase, (mono)dehydroascorbate reductase and others (for review see Halliwell, 1984; Young, 1991; Polle et al., 1990; Siefermann-Harms, 1994; Kranner and Lutzoni, 1999). Other compounds such as flavonoids, sugars, polyols, proline and polyamines probably also have antioxidant properties (for review see Smirnoff, 1993). In addition, in higher plants phenolic compounds can act as antioxidants (Rice-Evans et al., 1996), and in lichens, antioxidant activity of phenolic lichen products was reported by Hidalgo et al. (1994). However, it is not known if these secondary products have antioxidant function in fungal cells before being secreted and exposed extracellularly.

The xanthophyll cycle is a well known mechanism that protects photosystems against excess light by facilitating the thermal dissipation of excess light energy, thus avoiding formation of singlet oxygen (Siefermann-Harms, 1990; Demmig-Adams and Adams, 1996; Kranner and Lutzoni, 1999). In the xanthophyll cycle the zeaxanthin formed from violaxanthin, via the intermediate antheraxanthin, is thought to act as a protective pigment. Excess energy is utilised to remove two epoxy groups in violaxanthin resulting in lengthening of the conjugated system of double bonds from 9 in violaxanthin to 10 in antheraxanthin to 11 in zeaxanthin (Siefermann-Harms, 1977; Demmig et al., 1987; Eskling et al., 1997; Kranner and Lutzoni, 1999).

High irradiation and desiccation both induce formation of free radicals and are therefore extremely stressful to plants, and even more when these two factors are in combination. The aim of this study was to investigate whether the xanthophyll cycle is of importance in desert lichens, which are often exposed to a combination of extreme desiccation and irradiation. In this paper we report an experiment to investigate the xanthophyll cycle pigments in the desiccation tolerant desert lichen *Ramalina maciformis* (Delile) Bory during desiccation and rehydration. To determine whether the xanthophyll cycle operates in response to desiccation alone, we carried out two experiments, exposing lichens to constant light and constant dark respectively. In addition, we studied responses of lutein, neoxanthin, chlorophyll a and b, α- and β-carotene.

2. Materials and Methods

Lichen material

Ramalina maciformis (Delile) Bory was collected in the end of August 1998 in Israel, Negev south of Be'er Sheva, 7 km north of Sedé Boquer growing on rocks (leg. Scheidegger and Lutzoni). *R. maciformis* is a green alga-containing lichen with *Trebouxia* sp. as photobiont. After transporting the lichens to our lab, thalli were exposed to ambient air humidity (c. 70% relative air humidity) for one day prior to the experiment which was started 12 days after collection. Half of the material was kept in the dark while the other part was exposed to a day-night cycle.

Experimental

R. maciformis thalli with c. 17% water content (g 100 g^{-1} DW, as collected in the natural habitat) were used for desiccation and rehydration experiments. Two experiments were performed at 21°C. The first experiment was carried out in constant darkness, in the second, lichens were exposed to constant light (500 µmol photons m^{-2} s^{-1} PAR). Lichens were desiccated for 30 d in a desiccator over silica gel at 2% relative air humidity. During desiccation, samples were taken after 1, 3, 6 and 30 d (n=5). Rehydration experiments were carried out with thalli desiccated for 6 d ("short-term") and 30 d ("long-term"), respectively, by exposing them to air of 100% relative humidity. During rehydration, samples were taken after 0.5, 1, 2 and 12 h (n=5). Desiccated and rehydrated lichen samples were frozen in liquid nitrogen and stored in a deep freezer (–80°C) or freeze-dried immediately. Freeze-drying and further sample treatment was performed according to Pfeifhofer et al. (in press): after each period of desiccation and rehydration, samples were instantly frozen in liquid nitrogen and either stored at –80°C until freeze-drying, or freeze-dried immediately. Afterwards, the freeze-dried thalli were ground to a homogenous powder with a ball mill, and the powder was stored in humidity-proof vials at –25°C until analyses.

Analytical procedures

For one sample, 100 mg of the freeze-dried lichen powder was extracted in acetone containing 0.1% N-ethyldiisopropylamine. This organic base can prevent pigment destruction during extraction caused by secondary lichen products (Pfeifhofer et al., in press).

Pigments were identified and quantified using an HPLC gradient-method as described by Pfeifhofer et al. (in press). The HPLC-system consisted of two

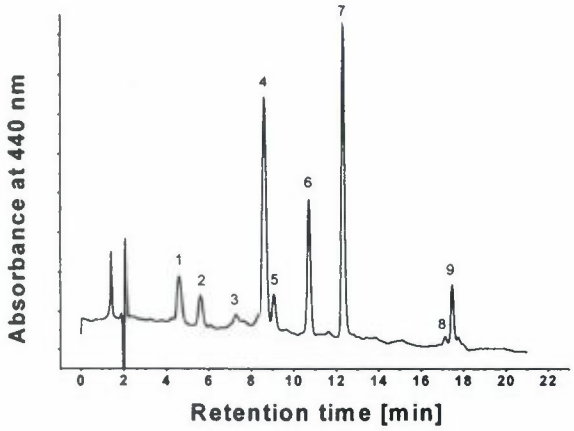

Figure 1. HPLC separation of chlorophylls and carotenoids of a *Ramalina maciformis* extract. 1, neoxanthin; 2, violaxanthin; 3, antheraxanthin; 4, lutein; 5, zeaxanthin; 6, chlorophyll b; 7, chlorophyll a; 8, α-carotene; 9, β-carotene.

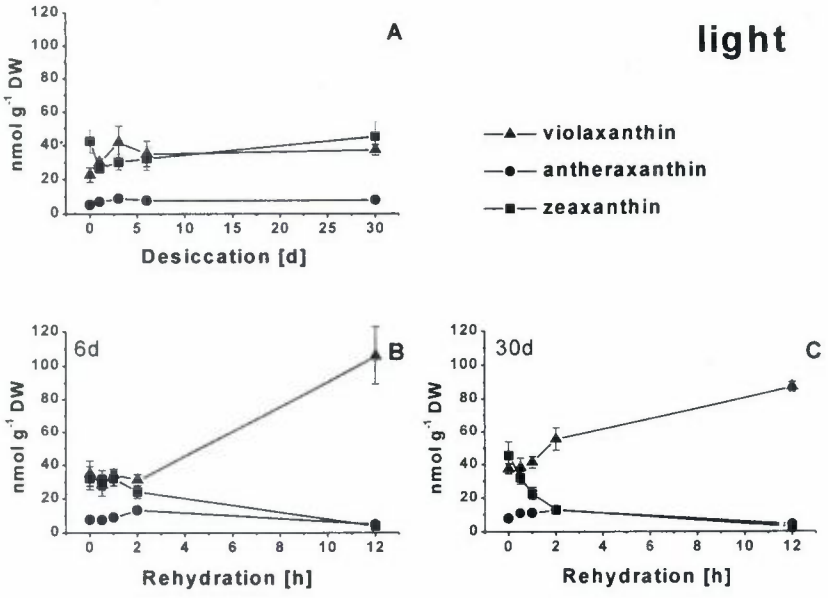

Figure 2. Xanthophyll cycle pigments during desiccation (A) of *Ramalina maciformis* in air of 2% relative humidity. Thalli desiccated for 6 d (B) and 30 d (C), respectively, were rehydrated in air of 100% relative humidity in constant light. Data represent mean ±SE (n=5).

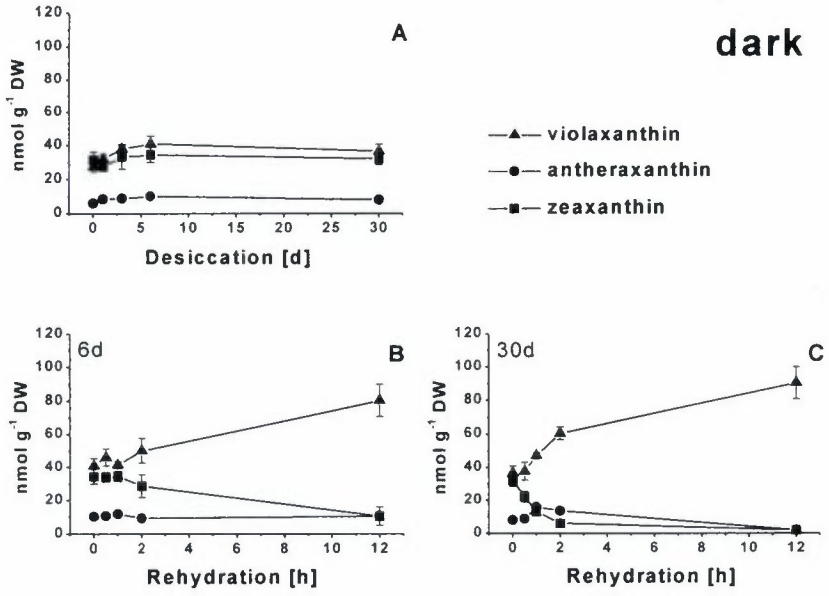

Figure 3. Xanthophyll cycle pigments during desiccation (A) of *Ramalina maciformis* in air of 2% relative humidity. Thalli desiccated for 6 d (B) and 30 d (C), respectively, were rehydrated in air of 100% relative humidity in the dark. Data represent mean ±SE (n=5).

Knauer HPLC Pumps 64, a solvent mixer, a cooled (4°C) autosampler (Basic Marathon from Spark Holland), a pre-packed 5 µm ODS-2 column (250 × 4 mm i.d.) and pre-column (10 × 4 mm i.d.) from Spherisorb, and a variable wavelength detector (Milton Roy SpectroMonitor 3100). For integration a gradient controlling software package from Knauer was used.

Settings: Solvent A, acetonitril:methanol:water = 100:10:5 (v/v/v); solvent B, acetone:ethylacetate = 2:1 (v/v); linear gradient from 10 to 70% B within 18 min, then 4 min 70% solution B (until elution of β-carotene); after equilibrating the column with 10% solvent B, the next run was started 30 min after the previous injection; flow rate = 1 ml min^{-1}. Absorbance of column effluents was measured at 440 nm. Using this system, adequate resolution, even of the xanthophyll isomers lutein and zeaxanthin, has been achieved (Fig. 1).

Statistics

Unless indicated otherwise, data were tested for significance with two-way and three-way non-parametric analysis of variance using an enhanced Kruskal-

Wallis test with data alignment according to the method of Hildebrandt (in Bortz et al., 1990).

3. Results

The green algal lichen *Ramalina maciformis* contains the complement of plastid pigments (Fig. 1), typically found in green algae (Hager and Stransky, 1970; Goodwin, 1980). In freshly collected, air-dried thalli we found the following contents of pigments (all nmol g^{-1} DW±SE): chlorophyll a, 796±138; chlorophyll b, 204±37; β-carotene, 38±6; α-carotene, 4±1; lutein, 256±38; neoxanthin, 54±8; violaxanthin, 22±4; antheraxanthin, 5±0.7 and zeaxanthin, 43±7.

Responses of plastid pigments to desiccation

Exposing air-dried thalli to further desiccation reduced thallus water content from 17 to 5% (g 100 g^{-1} DW) after 30 d (Table 1). Desiccation did not affect the contents of chlorophyll a and b, α- and β-carotine, lutein and neoxanthin, and their amounts did not differ significantly in thalli desiccated in the dark as compared to thalli exposed to constant light (data were tested for significance with the enhanced Kruskall-Wallis test).

Responses of plastid pigments to rehydration

After desiccation for 6 d thalli contained 8% water, and after 30 d 5%. During rehydration in air of 100% relative humidity water content increased significantly faster and to higher values in thalli desiccated for 30 d rather than 6 d (Table 1).

Rehydration caused a pronounced increase in violaxanthin ($P<0.001$) and a simultaneous decrease in zeaxanthin ($P<0.001$), irrespective of whether samples had been exposed to constant light or dark. Within 12 h of rehydration the violaxanthin content of lichens desiccated for 6 d and exposed to constant light increased by 204% as compared to non-rehydrated thalli, while the zeaxanthin content decreased by 88% (Fig. 2). Table 2 lists increases in violaxanthin and decreases in zeaxanthin contents within 12 h of rehydration. Note that when lichens had been rehydrated in constant light the amount of violaxanthin increase was significantly higher than the amount of zeaxanthin decrease in thalli desiccated for 6 h, whereas for all other treatments no statistically significant differences between the amounts of violaxanthin increase and zeaxanthin decrease were found.

Table 1. Water content (g 100 g^{-1} DW) during desiccation, and during rehydration following short-term (6 d) and long-term (30 d) desiccation of *Ramalina maciformis* in constant light. Rehydrating thalli in constant dark did not result in significantly different thallus water contents. Data represent means ±SE (n=5).

Desiccation		Rehydration of short-term desiccated thalli		Rehydration of long-term desiccated thalli	
Duration (d)	Water content (%)	Duration (h)	Water content (%)	Duration (h)	Water content (%)
0 (air dried)	17±1	0 (non-rehyd.)	8±0	0 (non-rehyd.)	5±2
1	12±1	0.5	17±1	0.5	23±1
3	6±1	1	18±1	1	25±1
6	8±0	2	22±2	2	27±1
30	5±2	12	29±1	12	43±7

Table 2. Amounts of violaxanthin increase and zeaxanthin decrease, calculated as differences between contents of non-rehydrated thalli and thalli rehydrated for 12 h of *Ramalina maciformis* following short-term (6 d) and long-term (30 d) desiccation. Data represent means ±SE (n=5). Differences between means were tested for significance with Mann-Whitney U test. For significant differences ($P<0.01$) between the amounts of violaxanthin increase and zeaxanthin decrease the letter 'a' is used. Significant differences indicate that in addition to the conversion of zeaxanthin to violaxanthin, *de novo* synthesis of violaxanthin occurred.

Sample treatment	Previous desiccation (d)	Violaxanthin increase (nmol g^{-1} DW)	Zeaxanthin decrease (nmol g^{-1} DW)
Constant light	6	70±12 [a]	28±5 [a]
	30	50±4	42±8
Constant dark	6	39±10	24±6
	30	54±13	30±4

The contents of β-carotene, lutein and neoxanthin did not change during rehydration and no significant differences between light and dark, and/or between rehydration following short- or long-term desiccation could be observed. Thalli rehydrated in the dark contained slightly more chlorophyll (chlorophyll b: $P<0.002$; chlorophyll a: $P<0.003$) and less α-carotene ($P<0.002$) than thalli rehydrated in the light, and this was found for thalli rehydrated after short- and after long-term desiccation.

4. Discussion

Desiccation did not affect the chlorophyll content of *R. maciformis*, indicating that this lichen belongs to the homoiochlorophyllous desiccation-tolerant group of photoautotrophic organisms. These preserve their photosynthetic apparatus with its pigments during desiccation in a state from which it can recover quickly and fully at rehydration (Tuba et al., 1993; Sherwin and Farrant, 1996). Lange (1969) reported that *R. maciformis* recovers maximum photosynthetic activity at thallus water contents of 60% when thalli are wetted after desiccation. Thalli that contained 30% water already showed 50% of maximum photosynthesis.

Desiccation of air-dried thalli did not affect contents of xanthophyll cycle pigments in *R. maciformis* irrespective of whether thalli have been exposed to constant light or dark (Figs. 2A and 3A). This is similar to results of Jensen et al. (1993), who did not find changes in xanthophyll cycle pigments in desiccated *Lobaria pulmonaria* thalli when exposing them to varying light intensities. Jensen et al. (1999) assumed an 'on/off-switch' for photosynthetic light reactions triggered by the water potential. Accordingly, we believe that our experimental conditions probably did not allow a conversion from violaxanthin to zeaxanthin during desiccation. At water contents between 10 and 15%, *R. maciformis* does not display photosynthetic activity (Lange, 1969). Air-dried thalli of *R. maciformis* only contained 17% water at the beginning of the experiment (Table 1), thus the water potential probably underwent the critical point of the supposed 'on/off-switch' and presumably thalli were too dry to allow any xanthophyll cycle activity during desiccation.

However, when dehydrating thalli with high initial water content, several authors reported formation of zeaxanthin during desiccation of cryptogams. The existence of a photoprotective mechanism with involvement of xanthophyll cycle pigments in non-radiative dissipation of desiccation-induced excess energy was discussed by Calatayud et al. (1997) who found increased antheraxanthin and zeaxanthin levels during dehydration of the lichen *Parmelia quercina*. Deltoro et al. (1998) reported an increase in zeaxanthin in dehydrated thalli of the desiccation tolerant liverwort *Frullania dilatata*. These authors assumed that while CO_2 fixation and therefore ATP consumption are decreased at low water content, continued electron flow gives rise to acidification of the thylakoid lumen thus inducing antheraxanthin and zeaxanthin synthesis. Moreover, they correlated the interaction of lumen acidity and de-epoxidised xanthophylls with enhanced non-photochemical quenching (NPQ). Furthermore, Csintalan et al. (1999) found a large increase in NPQ as desiccation tolerant mosses dried. That increase was prevented by dithiothreitol, which was used as an inhibitor of the xanthophyll cycle. By contrast, a recent report of Leipner et al. (2000) indicates that increased

zeaxanthin content is not necessarily correlated with thermal energy dissipation during photooxidative stress in maize leaves.

During rehydration, we interestingly found a pronounced increase in violaxanthin that was independent of light and of duration of previous desiccation (Figs. 2B,C and 3B,C). This is partly consistent with the results of Chen and Lai (1996) who reported fluctuations in violaxanthin contents during desiccation and rehydration under constant light of cultures of a desiccation tolerant strain of the terrestrial green alga *Chlorella* sp. In this alga, during the first 5 d of rehydration, violaxanthin decreased corresponding with a decrease in zeaxanthin. When growth resumed between 5 and 8 d, violaxanthin increased while zeaxanthin remained unchanged, indicating *de novo* synthesis of violaxanthin, similar to our findings in short-term desiccated thalli of *R. maciformis* when exposed to constant light (Fig. 2B and Table 2). Jensen et al. (1993) also found conversion from zeaxanthin to violaxanthin when storing wet *Lobaria pulmonaria* thalli in darkness, but in contrast to our results, they found that light treatment induced formation of zeaxanthin.

An increase in violaxanthin, either due to conversion of zeaxanthin (Figs. 2B,C and 3B,C), or *de novo* synthesis (Fig. 2B and Table 2), could perhaps be part of a rehydration-phase protection mechanism and interpreted as an adaptation to multiple stresses. This is supported by the finding that the combination of desiccation and light induced significant ($P<0.01$) *de novo* synthesis of violaxanthin in short-term desiccated thalli (Table 2). It remains unclear why no significant violaxanthin synthesis was found in long-term desiccated thalli exposed to constant light. Possibly, violaxanthin-synthesising enzymes were damaged by the combination of constant light and long-term desiccation, or the lichens were in a physiologically inactive status.

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