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RAPID COMMUNICATION

Lichen Substances Prevent Lichens from Nutrient Deficiency

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Abstract The dibenzofuran usnic acid, a widespread cortical secondary metabolite produced by lichen-forming fungi, was shown to promote the intracellular uptake of Cu^{2+} in two epiphytic lichens, *Evernia mesomorpha* and *Ramalina menziesii*, from acidic, nutrient-poor bark. Higher Cu^{2+} uptake in the former, which produces the depside divaricatic acid in addition to usnic acid, suggests that this depside promotes Cu^{2+} uptake. Since Cu^{2+} is one of the rarest micronutrients, promotion of Cu^{2+} uptake by lichen substances may be crucial for the studied lichens to survive in their nutrient-poor habitats. In contrast, study of the uptake of other metals in *E. mesomorpha* revealed that the intracellular uptake of Mn^{2+}, which regularly exceeds potentially toxic concentrations in leachates of acidic tree bark, was partially inhibited by the lichen substances produced by this species. Inhibition of Mn^{2+} uptake by lichen substances previously has been demonstrated in lichens. The uptake of Fe^{2+}, Fe^{3+}, Mg^{2+}, and Zn^{2+}, which fail to reach toxic concentrations in acidic bark at unpolluted sites, although they are more common than Cu^{2+}, was not affected by lichen substances of *E. mesomorpha*.

Keywords Lichenized Ascomycetes · Dibenzofurans · Depsides · Metal homeostasis · Copper

Introduction

Lichens are known for their ability to cope with extreme environments. They can adapt to extreme temperatures, drought, inundation, salinity, high concentrations of heavy metals (Nash 2008), or even survive in outer space (Sancho et al. 2007). Another outstanding character of lichens is tolerance to nutrient-poor environments. Mechanisms that enable a lichen to deal with shortages of nutrients have been poorly scrutinized, although most cannot compete with vascular plants at well-supplied sites.

Lichen substances are a chemically diverse group of more than 800 mostly phenolic compounds largely specific to lichen-forming fungi. They recently were shown to specifically inhibit the intracellular uptake of toxic amounts of transition metals, whereas other metals absent at toxic concentrations from the environment of the lichen could pass (Hauck 2008). Due to the high specificity of the role of lichen substances in metal tolerance, we tested the hypothesis that the opposite effect, the promotion of the uptake of metals needed as micronutrients, may be realized in the lichen symbiosis in nutrient-poor environments. We studied Cu^{2+} uptake in lichens with usnic acid (UA), a widespread cortical dibenzofuran in lichen-forming fungi. This example was selected, as Cu^{2+} is, after Mo, usually the rarest micronutrient in the microhabitats of epiphytes of nutrient-poor bark (Gauslaa 1995). Moreover, UA forms complexes with Cu^{2+} (Takani et al. 2002). We selected two UA-producing epiphytic lichens characteristic of nutrient-poor, acidic bark, *Evernia mesomorpha* Nyl. and *Ramalina menziesii* Taylor. The former lichen produces the depside divaricatic acid (DA) in addition to UA, whereas the latter lichen only synthesizes UA.

Methods and Materials

Lichens were collected in Mongolia (Khentey, Eroo, Khonin Nuga; *E. mesomorpha*) and the USA (Oregon, Benton County, Porter Lake; *R. thrausta*). Thalli were

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stored in air-dry condition at −18°C in the dark before the experiment. After thawing, extracellular lichen substances were extracted with acetone from one half of the lichen thalli. Samples were submersed in acetone (4×10 min). The efficacy of this treatment at removing lichen substances was controlled by high-pressure liquid chromatography by using a reverse-phased column and gradient elution. Viability of lichen thalli was controlled by measuring the chlorophyll fluorescence yield (Φ_2) of light-adapted samples at photosystem II after the acetone treatment with a PAM-2100 chlorophyll fluorometer (Walz, Effeltrich, Germany).

Five pieces of the fruticose thalli of up to 5-cm length were used in each replicate sample and combined on a Petri dish with a moist cellulose filter. For acclimatization, Petri dishes with thalli were stored in a growth chamber for 2 days at 80% RH, a day temperature (for 13 h daily) of 13°C with a photon flux density of 30 μmol m^{-2} s^{-1}, and a night temperature of 10°C. Lichens then were exposed for 30 min to 50 ml of 20 μM $CuCl_2$, which is typical of the microhabitats of the studied lichens under unpolluted conditions on a shaker at pH 4. The uptake of Fe^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} from chloride salt solutions was studied in *E. mesomorpha* in comparison. These salts were applied at a higher concentration (100 μM) than $CuCl_2$ because their concentrations in stemflow, throughfall, and bark, which form important nutrient sources for epiphytes, are higher than that of Cu^{2+} (Gauslaa 1995). Moreover, these metals cause less membrane damage than Cu^{2+}, which would impair the experiment. After exposure to the metal solution, the extra- and intracellular cations were sequentially extracted. For this purpose, samples were shaken with deionized water (2×20 ml) to remove free apoplastic ions. Metal ions bound to hydroxylic or carboxylic exchange sites of the cell wall were exchanged by shaking samples with $NiCl_2$ (2×20 ml). Afterwards, all samples were shaken with acetone (2×20 ml) to remove metal ions potentially bound to extracellular lichen substances. Samples were dried at 105°C, homogenized, and digested with 65% HNO_3 in order to analyze intracellular concentrations of Cu, Fe, Mg, Mn, or Zn with atomic absorption spectrometry (AAS Vario 6, Analytik Jena, Germany). The measuring error inherent to the AAS amounted to <1%.

Statistical analyses were computed with SAS 6.04 software (SAS Institute Inc., Cary, NC, USA). All data are given as arithmetic means±standard error and were tested for normal distribution with the Shapiro–Wilk test. Samples were tested for significant differences with Student's *t* test for pairwise comparisons.

Results and Discussion

Samples containing their natural content of lichen substances took up significantly more Cu^{2+} than acetone-extracted samples, both in *E. mesomorpha* (*t* test, $P \leq 0.01$, $df=4$) and in *R. menziesii* ($P \leq 0.05$; Fig. 1a, b). The promotion of metal uptake was highly specific for Cu^{2+}. Uptake of Fe^{2+}, Fe^{3+}, Mg^{2+}, and Zn^{2+} in *E. mesomorpha* was not influenced by lichen substances. Intracellular uptake of Mn^{2+} was even reduced ($P \leq 0.05$; Fig. 1c). This makes sense, as peak concentrations of Mn^{2+} leached from the canopy are known to limit the abundance of lichens on acidic bark (Hauck and Paul 2005), as has been found in conifers and some broad-leaved trees such as beech and oak. Hence, reduced uptake broadens the ecological niche of lichens, although Mn^{2+} is essential in minor amounts. Partial inhibition of Mn^{2+} uptake by lichen substances is already known from epiphytic lichens at Mn^{2+}-rich sites (Hauck 2008).

Stronger promotion of Cu^{2+} uptake in *E. mesomorpha* than in *R. menziesii* (Fig. 1) suggests that DA in addition to UA could contribute to Cu^{2+} uptake. However, although members of the same family (Parmeliaceae), both lichens differ in their morphology, which is also likely to affect element uptake. Complex formation of UA and Cu^{2+} in the pH range preferred by UA-producing lichens (pH 3.5–6;

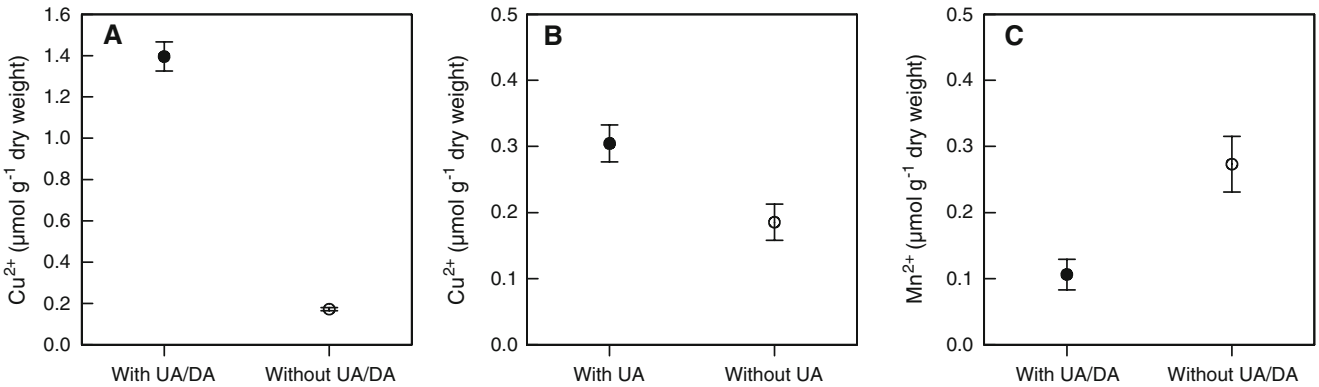

Fig. 1 Intracellular concentrations of Cu^{2+} in **a** *E. mesomorpha* and **b** *R. menziesii* and **c** Mn^{2+} in *E. mesomorpha* with and without their natural content of lichen substances after exposure to $CuCl_2$ or $MnCl_2$ solution for 30 min ($N=5$). *Error bars* indicate standard error

Takani et al. 2002; Hauck and Jürgens 2008; Hauck et al. 2009) suggests that these complexes are involved in the observed promotion of Cu^{2+} uptake. Whether such lipophilic complexes directly cross the phospholipid membrane (as proven for uncomplexed UA; Abo-Khatwa et al. 1996; Hauck and Jürgens 2008) or Cu^{2+} ions are transferred to transporters is unclear. Given the multitude of compounds and their widespread ability to interact with metal ions (Hauck and Huneck 2007), it is likely that promotion of nutrient uptake will be seen in other lichen symbioses. Recent UV-spectroscopic studies in yellow and orange lichen substances, including UA, suggested that promotion of metal uptake by lichen substances might be widespread (Hauck et al. 2009). Lichen compounds that occur in lichens of nutrient-poor sites form complexes with metal ions precisely at the pH ranges preferred by the respective lichens. Lichens growing under pH conditions, at which lichen substances do not bind to metals, are limited to substrata rich in mineral nutrients (Hauck et al. 2009). Research is necessary to elucidate the extent to which lichen substances are involved in the metal homeostasis of lichens.

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