

Pentose-phosphate pathway as the possible background of resaturation respiration in the desiccation-tolerant lichen *Cladonia convoluta*

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Abstract. Respiration rates in the re-wetted desiccated thalli of a desiccation-tolerant lichen *Cladonia convoluta* were measured during rehydration and revival. Treatment with bromoxynil as uncoupler of oxidative phosphorylation showed that the high intensity of resaturation respiration rate is not entirely caused by the uncoupled mitochondrial membrane. Treatments with KCN or absence of O₂ obtained by applying N₂ resulted in the same effect indicating the possible role of the pentose-phosphate shunt in the boosted CO₂ efflux of the rehydrating and reviving desiccated lichen thalli.

Keywords: basic respiration, bromoxynil, *Cladonia*, uncoupling, desiccation tolerance, mitochondrion, potassium cyanide, rehydration, wetting burst

Introduction

Desiccation tolerant plants can survive the loss of 80-95% of their cell water, so that the plants appear completely dry and no liquid phase remains in their cells; after a shorter or longer period in the desiccated state, they revive and resume normal metabolism when they are re-moistened (Proctor et al., 2002). In a dehydrated desiccation tolerant plant an intense CO₂

emission can be observed in the very first minutes after re-wetting, which is considered to be a physical process; i.e. water replaces CO₂ molecules absorbed on the cell walls (Brown et al., 1983). However, this wetting burst is followed for a few hours by a respiration which has been termed resaturation respiration and which has a higher intensity than that of constantly metabolically active lichens (Smith & Molesworth, 1973). This elevated respiration does not serve the replenishment of the ATP pool because the latter is already completed within 5-10 minutes after rewetting (Krochko et al., 1979). Gordy and Hendrix (1982) considered the resaturation respiration of mitochondrial origin because they could eliminate completely the O₂ uptake during this phase with the simultaneous usage of cyanide and salicylhydroxamic acid. The increasing evidence of membrane destruction in desiccated lichens led to the conclusion that resaturation respiration is a result of the uncoupled inner mitochondrial membrane (Farrar & Smith, 1976).

The initially high rehydration respiration rate is also occurring in the higher/flower desiccation-tolerant higher flowering plants, like in the monocot *Xerophyta scabrida* where it lasts up to 30 hours (Tuba et al., 1994). In the lichens and mosses the resaturation respiration lasts only from few minutes up to 5 h (e.g. Smith & Molesworth 1973; Bewley et al. 1978).

In order to reveal the biochemical background of the resaturation respiration, we treated a highly desiccation tolerant cryptogamic species, the lichen *Cladonia convoluta* (see: Tuba et al., 1996) with uncouplers, with potassium cyanide (KCN) and bromoxynil (Kerr and Wain, 1964 and Hock et al., 1995) or we deprived them of oxygen by applying inert N₂ gas. Our aim was to identify the possible cause of the boosted CO₂ efflux during the rehydration of the desiccated *C. convoluta* lichen thallus.

Materials and Methods

The foliose green algae lichen thallus *Cladonia convoluta* (Lam.) P. Cout. were used in the experiment after being collected from a semi-desert sand grassland (Fekete et al., 1997) near Fülöpháza (Hungary, Great Plain). The samples were cleaned by rinsing in deionized water and placed on wet filter paper. The upper green part of the moss and the separated healthy lobes of lichen thallus were used. Prior to desiccation plants spent 24 hours in a metabolically active state (20 °C, 100% Rh, 200 μM m⁻² sec⁻¹ PhAR for 14 hours). Slow desiccation was carried out on the next day when Rh was lowered to 70%. For rehydration deionized water (control, N₂-treatment), 10⁻³ M KCN, and 10⁻³ M bromoxynil (4-hydroxy-3,5 dibromo benzonitrile) were applied. Rehydration was conducted in small Plexiglas chambers (20 °C, 100% Rh, 200 μM m⁻² sec⁻¹ PhAR). CO₂ gas exchange measurements were carried out according to Tuba et al. (1996) in five

replications with a ADC LCA2 (Hoddesdon, UK) infrared gas analyser in the first 30 minutes after rewetting.

Results and Discussion

The basic respiration rate of the continuously wet lichen *C. convoluta* was 2.6 ± 0.29 . However, the resaturation respiration rate of the wetted lichen was three times higher (Fig. 1.). The high intensity rehydration ('resaturation') respiration was terminated within 30 minutes and during the remaining 90 min of the experiment the respiration declined to a steady level (data not shown).

Treatment with bromoxynil, an uncoupler of oxidative phosphorylation, increased this already high CO_2 emission by 66% indicating that the extraordinarily massive CO_2 efflux does not come entirely from the mitochondria, which has lost adenylate control due to uncoupled inner membranes. Nitrogen exposure (anaerobic conditions) and KCN treatment resulted in a surprising coincidence. They both decreased CO_2 emission rate by 30% in the lichen. This fact suggests that there is a component, which is non-sensitive to both cyanide and also oxygen deprivation. The only biochemical process, which can produce a significant amount of CO_2 in not senescing plant cells under anaerobic conditions, is the pentose-phosphate pathway. This pathway can play an essential part in the restoration of the plant's redox status by producing NADPH (Kranter & Grill, 1994).

This observation also infers that the alternative mitochondrial pathway is not engaged after rehydration of the desiccated tissues, because CO_2 emission was not decreased more by the lacking of O_2 than by cyanide, which clogs cytochrome pathway selectively. Due to the Bahr-Bonner model (Lambers, 1982), which considers the alternative oxidative pathway as an overflow, operating the alternative pathway would not make too much sense in desiccation tolerant plants spending its resources for recovery from the desiccated stage.

Based on the above findings we would like to emphasise that the preponderance of resaturation respiration of the desiccated lichen thalli probably originates from the pentose-phosphate pathway.

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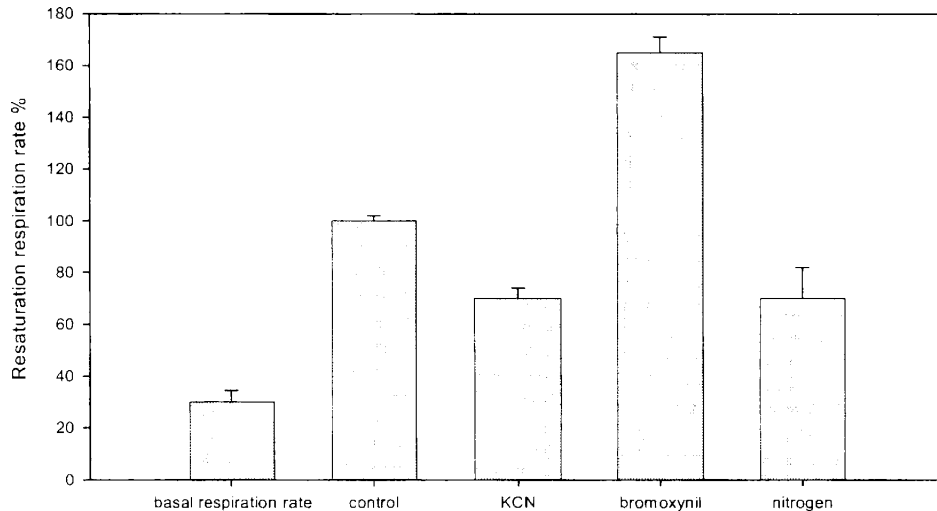


Fig. 1. Rates of resaturation respiration and basal respiration (as percentage of the control plants) of the rehydrating desiccated lichen *C. convoluta* thalli in the control, and in the KCN (10^{-3} M), bromoxynil (10^{-3} M) and N_2 treated lichen ($n = 5$, bars represent standard deviations). Note that the rate of resaturation respiration was four times higher than the so-called basal (basic) respiration.

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