Long-term effect of elevated CO2 on spatial differentiation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in Norway spruce canopy

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Abstract

Total in vitro activity of RuBPCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme was assayed spectrophotometrically by the continuous measurement of 3-phosphoglycerate-dependent NADH oxidation in a coupled enzyme system. RuBPCO activities were found in the ranges 1.01–2.76 and 1.23–3.10 µmol(CO2) m-2 s-1 in current Norway spruce needles growing in ambient (AC) and elevated (EC) CO2 concentration, respectively. RuBPCO activity in AC needles from the upper layer (U) was 11–15 % higher compared to those from the middle (M) layer, and even 44–56 % higher compared to the lower (L) layer of spruce crown. Over the vegetation season, we observed a highly significant decrease of RuBPCO activity in the EC-U needles from 3.10 (July) to 1.60 (October) µmol(CO2) m-2 s-1 as a consequence of downward feedback regulation. Moreover, this down-regulation was not caused by a non-specific decrease in total leaf nitrogen content.

Additional key words: down-regulation; nitrogen; photosynthesis; Picea abies; sun and shade needles; vertical profile.

Introduction

RuBPCO (ribulose-1,5-bisphosphate carboxylase/oxygenase, EC 4.1.1.39) enzyme is the most widespread and perhaps the most remarkable protein on the Earth because it is the only one which provides a significant link between the pools of inorganic and organic carbon in the biosphere. This enzyme catalyses carboxylation of D-ribulose-1,5-bisphosphate (RuBP), the first step of the Calvin cycle in competition with oxygenation of RuBP that leads to the photorespiratory pathway. Catalytic effectiveness of RuBPCO is low both in terms of its catalytic rate ($k_{cat} = 2–12$ s-1) and of its substrate concentration [$k_{cat}/K_M(CO_2) = 5–40 \times 10^7$ mol-1 m3 s-1] (Roy and Andrews 2000). Therefore, photosynthetic organisms invest up to 50 % of soluble leaf protein (Eichelmann and Laisk 1999) in RuBPCO to support acceptable rates of photosynthesis. RuBPCO must be reversibly activated with CO2, Mg2+, and RuBPCO activase before catalysis can occur. Hence, carbon dioxide has two direct biochemical effects on plants. It acts both as the activator and the substrate of RuBPCO enzyme.

Continuous increase of atmospheric CO2 concentration ([CO2]) led to extensive research over the last decades. Elevated [CO2] (EC) strongly affects photosynthesis and growth of many plants, especially C3 plants which constitute more than 90 % of terrestrial species. Short-term exposure of higher plants to EC usually increases photosynthetic CO2 uptake, since an increased CO2 partial pressure stimulates carboxylation and suppresses oxygenation. Photosynthetic responses to long-term EC are generally more variable (reviewed e.g. by Eamus and Jarvis 1989, Urban 2003).

The theoretical models (Luo et al. 1994, Griffin and Seemann 1996) and many experimental results (Ceulemans and Mousseau 1994, Sage 1994, Urban and Marek 1999, Urban et al. 2003) demonstrated that an adjustment of photosynthesis under EC is controlled by the interrelationships between biochemical and morphological feedback mechanisms. Over the time scale of...
hours to days, increased contents of specific saccharides (e.g. glucose, sucrose) lead, via hexokinase-related signal, to the repression of RuBPCO gene expression (Griffin and Seemann 1996) and a subsequent decrease of its content and activity (van Oosten and Besford 1996, Drake et al. 1997, Moore et al. 1999). Moreover, local phosphorus deficiency corresponds to RuBPCO de-carboxylation owing to reduced activity of RuBPCO activase (Portis 1990). These biochemical adjustments, which are often termed acclimation or down-regulation, reduce photosynthetic capacity. Morphological adjustments, most often observed as increased mesophyll tissue growth (Vu et al. 1989), are indicated by changes in leaf mass per unit area and tend to increase photosynthetic capacity, i.e. up-regulation. The mutual relations among these two processes at a given [CO$_2$] determine the net photosynthetic rate ($P_n$).

Strong vertical differences of photosynthetically active radiation (PAR), typical within close forest stands, lead to the changes of both structure and function of the assimilatory apparatus (Hättenschwiler 2001). Because PAR is the limiting factor for shade acclimated leaves, a substantial amount of photosynthetic resources must be invested in the synthesis and maintenance of light-harvesting complexes, while large amounts of electron transport components, ATP synthase, or the stromal CO$_2$ fixation enzymes are not so required (Anderson et al. 1988, Priwitzer et al. 1998). On the contrary, sun adapted leaves are characterized by a high content of the cytochrome b/f complex, plastoquinone, plastocyanin, ferredoxin, and ATP synthase which support fast rates of electron transport and photophosphorylation (Anderson et al. 1988, Špunda et al. 1998). Also, formation of upward vertical gradients of important macronutrients, especially nitrogen, is typical for closed canopies (Hrdlička 1996). It tightly correlates with the different activities of important photosynthetic enzymes, including RuBPCO (Roy and Andrews 2000), and it subsequently leads to double values of maximal carboxylation rate, that reflect RuBPCO activity in vivo, for exposed comparing to shaded needles (Špunda et al. 1998, Šprtová and Marek 1999).

General objectives of this paper are (1) to determine spatial variability of RuBPCO activity within spruce canopy, and (2) to evaluate its change under the impact of EC.

**Materials and methods**

**Plants and experiment design:** The experiment was conducted in 2002 at the experimental site Bílý Kříž in Beskydy Mts. (Czech Republic, 49°30’N, 18°32’E, 908 m a.s.l.). Seventeen-year-old Norway spruce (*Picea abies* [L.] Karst.) trees have been grown since 1997 in two glass domes with ambient [350 µmol(CO$_2$) mol$^{-1}$; AC variant] or elevated [700 µmol(CO$_2$) mol$^{-1}$; EC variant] CO$_2$ concentration (for details see Urban et al. 2001). Both the total RuBPCO activity and N content were assessed in current needles taken from upper (U, 3$^{\text{rd}}$ whorl), middle (M, 6$^{\text{th}}$ whorl), and lower (L, 9$^{\text{th}}$ whorl) parts of tree crowns. Needles were sampled between 11:00 and 15:00 h at the following dates: 16$^{\text{th}}$ July (midday maximum temperature 13.8 °C, midday maximum irradiance 1 290 µmol m$^{-2}$ s$^{-1}$), 6$^{\text{th}}$ August (23.6 °C, 1 100 µmol m$^{-2}$ s$^{-1}$), 16$^{\text{th}}$ September (9.4 °C, 1 050 µmol m$^{-2}$ s$^{-1}$), and 10$^{\text{th}}$ October (5.6 °C, 150 µmol m$^{-2}$ s$^{-1}$). After determining needle fresh mass (*Sartorius*, Japan) and leaf area (*Li-3000A*, Li-cor, USA), samples (n = 3) for determining total leaf N content were dried to a constant mass at 104 °C, while samples (n = 6) for total RuBPCO activity assay were irradiated 5 min by saturating photosynthetically active radiation, PAR (>1 200 µmol m$^{-2}$ s$^{-1}$). To avoid any drought effects by this pre-treatment, the incident PAR was passing through the thermal filter and the room temperature was kept between 20 and 23 °C. After that, needle samples were immediately frozen in liquid nitrogen, transferred to Dewar vessels, and transported to the laboratory.

**RuBPCO extraction and assay:** RuBPCO protein was extracted from the needles and assayed for total activity using the techniques of Besford (1984), slightly modified by Hrstka et al. (2002). Approximately 0.3 g of needle tissue was homogenized in a chilled mortar with 0.1 g inert sand, liquid nitrogen, and 10 cm$^3$ extraction buffer composed of: 50 mM HEPES, 25 mM KHCO$_3$, 5 mM MgCl$_2$, 0.2 mM Na$_2$EDTA, 5 mM dithiothreitol, and 0.1 g of insoluble polyvinylpolypyrrolidone, all at pH 8.0. The homogenate was filtered through iced sintered glass R2 and the filtrate was assayed for total RuBPCO activity.

Total RuBPCO activity was assayed spectrophotometrically by the continuous measurement of 3-phosphoglycerate-dependent NADH oxidation in a coupled enzyme system based on the method of Lilley and Walker (1974). Reaction mixtures in cuvettes of 1.0 cm path length consisted of 30 mm$^3$ sample extract and 940 mm$^3$ assay solution containing 50 mM HEPES (pH 8.0), 25 mM KHCO$_3$, 20 mM MgCl$_2$, 0.2 mM Na$_2$EDTA, 5 mM dithiothreitol, 3.5 mM ATP, 0.35 mM NADH, 3.5 mM phosphocreatine, 67 nkat glyceraldehyde-3-phosphate dehydrogenase, 111 nkat 3-phosphoglyceric phosphokinase, and 178 nkat creatine phosphokinase. After 15 min of complete activation, 30 mm$^3$ of 13 mM RuBP was added to the sample/assay solution and changes in $A_{340}$ were measured at 25 °C using a Helios $\gamma$ (Spectronic Unicam, UK) spectrophotometer.

**Leaf nitrogen content** was determined after combustion by a CNS-2000 analyser (LECO Corp.).

**Statistics:** Because of small number of repetitions (n = 6), normal like distribution of the data sets was presumed. Scheffe test (ANOVA) was used to evaluate