Regulation of C₄-phosphoenolpyruvate carboxylase activity by ambient CO₂

Y. SAMARAS & Y. MANETAS
Laboratory of Plant Physiology, Department of Biology, University of Patras, Patras, Greece; *author for correspondence

Received 1 February 1988; accepted in revised form 13 April 1988

Key words: CO₂, light activation, PEPCase, Setaria verticillata

Abstract. Light activation of phosphoenolpyruvate carboxylase from the leaves of the C₄ plant Setaria verticillata (L.) is more pronounced at low CO₂ levels. The 2-fold activation observed at physiological ambient CO₂ becomes 3.64-fold at 5 μL/L and completely abolished above 700 μL/L. When the stomata close under the influence of abscisic acid at 330 μL/L CO₂, the extent of light activation is high (3.59-fold), probably because the increased diffusive resistance keeps the internal CO₂ at much lower levels. Under darkness, CO₂ and abscisic acid do not affect the extractable phosphoenolpyruvate carboxylase activity. Internal CO₂ levels may determine phosphoenolpyruvate concentration in the cytoplasm through the control of its utilization by phosphoenolpyruvate carboxylase. We have recently proposed (Samaras et al. 1988) that photosynthetically produced phosphoenolpyruvate could be an activator of the enzyme. It is therefore suggested that CO₂ indirectly affects the activation state of phosphoenolpyruvate carboxylase by controlling the levels of phosphoenolpyruvate which may act as an activator.

Abbreviations: PEPCase – phosphoenolpyruvate carboxylase, PEP – phosphoenolpyruvate, PAR – photosynthetically active radiation, G6P – glucose-6-phosphate, ABA – abscisic acid, MDH – malate dehydrogenase, PPDK – pyruvate, Pi, dikinase, CAM – Crassulacean Acid Metabolism

Introduction

Phosphoenolpyruvate carboxylase (PEPCase) is a key enzyme for C₄-photosynthesis, being responsible for the β-carboxylation of PEP to give oxaloacetate and Pi. Aspects of its structure and regulation have been recently reviewed (O’Leary 1982, Andreo et al. 1987). In most of the studies the enzyme appears as a homotetramer with Mr 400,000. It is activated in vitro by G6P, inhibited by oxaloacetate and malate and shows a broad maximum of activity at around pH 8. An early report on the light activation of PEPCase (Slack 1968) was largely overlooked, but the recent confir-
mation of these results (Karabourniotis et al. 1983 and 1985, Huber and Sugiyama 1986) revived interest in the in vivo regulation of the enzyme. Although a coherent picture has not yet emerged, association-dissociation phenomena (Walker et al. 1986, Wagner et al. 1987), phosphorylation of the day form (Nimmo et al. 1987), or a PEP-induced activation of the enzyme (Samaras et al. 1988) might be related to the higher activity and the altered kinetic properties of the day form.

If light activation of PEPCase has any physiological significance as a way of coordinating enzyme activity with photosynthetic rate, then factors affecting the latter might also influence the extent of activation. Indeed, day activity increases with irradiance (Karabourniotis et al. 1983, Nimmo et al. 1987) and with leaf temperature in the physiological (5–30°C) range (Samaras et al. 1988). In this paper we report that light activation of PEPCase is also influenced by the ambient CO₂ level.

Materials and methods

Plant material

Setaria verticillata (L.) Beauv. was grown from seeds in a walk-in growth chamber, with temperature, relative humidity and light/dark cycles of 30/20°C 40/70% and 13/11 h respectively. Irradiance at plant level was around 250 μmoles/m².s PAR, given by a mixture of fluorescent and incandescent lamps. All experiments were done with mature leaves from plants of four to six weeks of age.

Incubation under varying CO₂ levels and application of ABA

Mature leaves were cut from the plant 70 min before the onset of light, pre-weighed under a dim green safe light and floated with the less diffusive resistance side facing upwards, in small troughs with deionized water. The troughs were inserted in a temperature controlled leaf chamber maintained at 30°C and connected in series with an Infra-Red Gas Analyzer. In all cases, outside air was pumped and before entering the leaf chamber was treated as follows: a) CO₂ free air was produced by bubbling through two consecutive CO₂ traps (40% KOH); b) for intermediate levels between 0 and 330 μL/L CO₂, the CO₂ free air was enriched in CO₂ generated from a 80 mM NaHCO₃ solution dropped in 30% Trichloroacetic acid. Flow rate of NaHCO₃ and corresponding CO₂ levels were adjusted with a peristaltic pump; c) for CO₂ levels above 330 μL/L bubbling through KOH was omitted and outside air was enriched in CO₂ as previously described. In all cases, a 25 liter tank was inserted just before the entrance to the leaf chamber in