Regulation of RuBP carboxylase activity associated with photo-inhibition of wheat

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Abstract. Detached wheat leaves were illuminated in air until a steady rate of photosynthesis was established. Then the gas was changed to 1% O₂, 99% N₂ and after 2.5 h further illumination the capacity of the leaves for photosynthesis in air was decreased to approximately 50%. Measurement of RuBP carboxylase activity in extracts showed that inhibition of photosynthesis was accompanied by 70% inactivation of this enzyme. The capacity for photosynthesis and the activity of RuBP carboxylase were recovered when leaves were returned to normal air. Extracts of the leaves made when photosynthesis and carboxylase activity were low, recovered most of the lost carboxylase activity when supplemented with bicarbonate and magnesium ions. The time courses for activation and inactivation of the RuBP carboxylase in these experiments suggests the operation of a mechanism that has not yet been elucidated.

Introduction

A possible role for photorespiration, as an adjunct to the photosynthetic carbon reduction cycle, is to protect the photochemical apparatus at high light intensities when CO₂ is limiting [12]. With bean leaflets [15], inhibition of photosynthesis was observed upon exposure to high light intensities in the absence of CO₂ and when photorespiration was restricted by decreasing O₂ in the atmosphere. The inhibition of photosynthesis was accompanied by decreased photosystem II activity [4, 14, 16]. An apparently similar inhibition was observed in chilling sensitive plants [13] but this was shown to be dependent on the presence of oxygen during illumination. Weiss [21] found that preincubation of leaves or chloroplasts in the absence of CO₂ at temperatures above 20°C caused inhibition of photosynthesis and deduced from observations of the products of ¹⁴CO₂ fixation that an inactivation of RuBP carboxylase might be involved. Powles, Osmond and Thorne [16] suggested that decreases in carboxylase activity might be associated with the photo-inhibition they observed but provided no experimental evidence. Bahr and Jensen [3] and Heldt, Chon and Lorimer [5] showed that RuBP carboxylase present in isolated chloroplasts is only partly activated, especially from chloroplasts kept in darkness. Robinson, McNeil and Walker [17] and Leegood and Walker [7] obtained evidence with protoplasts contrary to this view. However, Mächler and Nösberger [10] have provided evidence for
variations in the degree of activation of RuBP carboxylase in intact leaves depending on environmental conditions experienced by the plants immediately before and during extraction.

A study of RuBP carboxylase purified from wheat leaves [9] shows that this enzyme, like that from other sources, becomes rapidly inactivated in the absence of CO₂ and Mg²⁺. Activity is restored during a few minutes of incubation with CO₂ and Mg²⁺. When deprived of CO₂ and Mg²⁺ for 24 h or after prolonged storage at low temperature a further inactivating process occurs in the enzyme from wheat that can only be reversed by prolonged incubation with CO₂ and Mg²⁺ at 20 to 25°C [9].

We report here photo-inhibition in wheat leaves under the conditions employed by Powles and Osmond [15]. We also demonstrate that the state of activation of RuBP carboxylase in the leaf is decreased. Since the nature of the inactivation may be significant for regulation of photosynthesis and photorespiration, we report also a preliminary investigation of inactivation in vivo and reactivation in vitro.

Materials and methods

Plant material

Wheat (Triticum aestivum cv. Kolibri) was grown in a mixture of peat and sand in pots in constant environment rooms, 4.1 x 2.7 m with reflecting walls, with 16 h photoperiods in 1.36 KW m⁻² fluorescent and 0.22 KW m⁻² tungsten lamps providing 600μE m⁻² s⁻¹ PAR at plant level. Minerals were provided from Hoagland solution with additional sodium nitrate to increase nitrate supply by a factor of 4. The third leaves of plants were used, when just fully expanded, 29 or 30 days after germination at day/night temperatures of 13°/10°C. Before harvesting, plants were kept in darkness overnight. Leaves were detached in dim light and immediately immersed in water. A 4 cm length was then cut from the middle region of each leaf. A group of five such leaf segments were transferred to small racks with their cut bases in water [6, 20].

The racks were then placed in water-jacketed leaf chambers, 10 cm³ in volume, described in detail by Arrabaca [1]. Gas mixtures were provided from steel cylinders through pressure-reducing valves and flow regulators, giving 450 ml min⁻¹ flow to each chamber. Leaf samples were illuminated by a tungsten halogen lamp giving 1000μE m⁻² s⁻¹ at the leaf surface.

Conditions for photo-inhibition

Photosynthesis by leaf samples was allowed to develop during 60 min illumination at 13°C with air passing through the leaf chambers. The air was then replaced by a gas mixture containing 1% oxygen, 99% nitrogen and this condition maintained for 2.5 h. To study recovery from the treatment, the gas mixture was then again switched back to air.