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Responses of Rubisco activity in vivo to light intensity and CO_2 in rice leaves

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Introduction

Rubisco is the key enzyme of photosynthesis and the most abundant leaf protein. Rubisco activity is thought to be a rate-limiting factor for the light-saturated rate of photosynthesis at present atmospheric CO_2 levels. In response to changes in light and CO_2 levels, however, the in vivo activity of Rubisco is modulated by reversible carbamylation or tight binding of 2-carboxyarabinitol 1-phosphate (CA1P) and other sugar-phosphate inhibitors to its catalytic sites. Rubisco activase mediates this modulation by removing sugar-phosphate inhibitors from the catalytic sites and by promoting carbamate formation. However, the relative contribution of each regulation (carbamylation or inhibitors) varies greatly among species.

The carbamylation of Rubisco in vivo can be deduced by the ratio of the activity measured immediately in extracts prepared rapidly to the activity measured following incubation with saturating concentration of CO_2 and Mg^{2+}. These measurements are called 'initial' and 'total' activities, and the ratio of the initial activity to total activity is termed the 'activation state' or 'carbamylation ratio'. Exactly speaking, however, this total activity is not a total activity, because it does not include the activity blocked by inhibitors such as CA1P. Therefore, total activity measurements often underestimate the potential activity of Rubisco. This means that such 'activation state' does not necessarily indicate 'true' activation state of Rubisco. Parry et al. (1997) reported the method for determining the potential activity of Rubisco after removing inhibitors with high concentrations of sulfate, and called the 'maximal activity'.

In this paper, we used transgenic rice plants with decreased Rubisco (40% wild type Rubisco) which had been transformed by *rbc*S antusense gene (Makino et al., 1997), and examined the responses of Rubisco activity in vivo to light and CO_2. Changes in 'initial', 'total' and 'maximal' activities of Rubisco were measured in response to light and CO_2. Rice plants belong to a species with extensive CA1P inhibition of Rubisco (Makino et al., 1994).

Materials and Methods

Rice (*Oryza sativa* L. cv Notohikari) was used, and the transgenic rice plants with decreased Rubisco were obtained by transformation with *rbc*S antisense gene (Makino et al., 1997). The transformant with wild-type 40% Rubisco (AS-71) was used (Makino et al., 2000). All plants were grown hydroponically in a greenhouse with supplemental illumination from five 500-W metal-halide lamps (700 μmol m^{-2} s^{-1} at the plant level). The greenhouse was controlled at 25˚C during the day and 20˚C at night. The uppermost, fully expanded leaves of the about 60-old plants were used for experiments.

Gas exchange and Chl fluorescence from PS II were simultaneously determined with an open gas-exchange system using a temperature-controlled chamber equipped with a fan. The

gas exchange system was previously detailed by Makino et al. (1988), and Chl fluorescence was measured with a PAM Chl fluorometer (Mini-PAM, Walz).

Rubisco activity was measured at 25˚C spectrophotometrically by coupling 3-PGA formation with NADH oxidation according to the method of Lilly and Walker (1974) modified by Sharkey et al. (1991). The initial, total and maximal activities were measured on the same extracts from the leaves. When the steady-state rate of photosynthesis was attained with the gas-exchange system, the leaf was rapidly frozen in liquid N_2. The frozen leaf was quickly homogenized in 50 mM Hepes/NaOH buffer (pH 8.0) containing 5 mM DTT and 20 mM $MgCl_2$. The initial activity was measured within 2 min of the start of extraction, and total activity was determined from the extracts after incubation with 20 mM $NaHCO_3$. The maximal activity was measured according to Parry et al. (1997). After incubation with 200 mM Na_2SO_4 at 4˚C for 30 min, the extracts were passed through a small column of Sephadex G-25. The eluate was incubated with 20 mM $MgCl_2$ and 20 mM $NaHCO_3$ at pH 8.0, and used for the assay. The amount of Rubisco protein was determined spectrophotometrically after formamide extraction of Coomassie brilliant blue R-250-stained subunit bands by SDS-PAGE. The activation state of NADP-malate dehydrogenase (MDH) was assayed according to Scheibe and Stitt (1988).

Results and Discussion

The CO_2 response of photosynthesis and Rubisco activity at 1500 μmol m^{-2} s^{-1} was first examined (Fig. 1). While the initial slope of photosynthesis to intercellular CO_2 pressure (Ci) was about 2-fold greater in the wild-type plants than in the *rbc*S antisense plants, the difference between the plants decreased with increasing Ci. The quantum yield of PS II (ΦPS II) was also greater in the wild-type plants. However, although ΦPS II in the wild-type plants reached maximum around atmospheric CO_2 levels, that in the antisense plants increased with increasing Ci. In contrast, NPQ in the antisense plants was higher over wide range of Ci. The true activation state of Rubisco (the ratio of the initial activity to maximal activity) maintained at high levels in both plants, but a small decrease was found below 10 Pa and above 60 Pa of Ci. In spite of the conditions of high light, a significant Rubisco inhibition was observed around high Ci in the antisense plants. The NADP-MDH activation was not correlated with Rubisco activation, but correlated with ΦPS II.

The light response of photosynthesis and Rubisco activity at 36 Pa CO_2 was next examined (data not shown). Whereas light-saturated photosynthesis was about 2-fold greater in the wild-type plants in the antisense plants, the initial slope of the light response curve was not different between the plants. This was caused by higher carbamylation of Rubisco (the ratio of the initial activity to total activity) in the antisense plants under low light conditions, and Rubisco inhibition was not different (no difference in total activities). The inhibition ratio of Rubisco activity at dark was about 80% in both plants, and decreased with increasing light intensity. No significant inhibition was found around light-saturation point. The NADP-MDH activation was not correlated with Rubisco activation, but correlated with ΦPS II.

In summary, although the carbamylation ratio of Rubisco was higher in the antisense plants under low light conditions, Rubisco activity in the antisense plants was relatively inhibited under elevated CO_2 conditions. Neither the carbamylation ratio nor true activation state of Rubisco was correlated with the NADP-MDH activation.

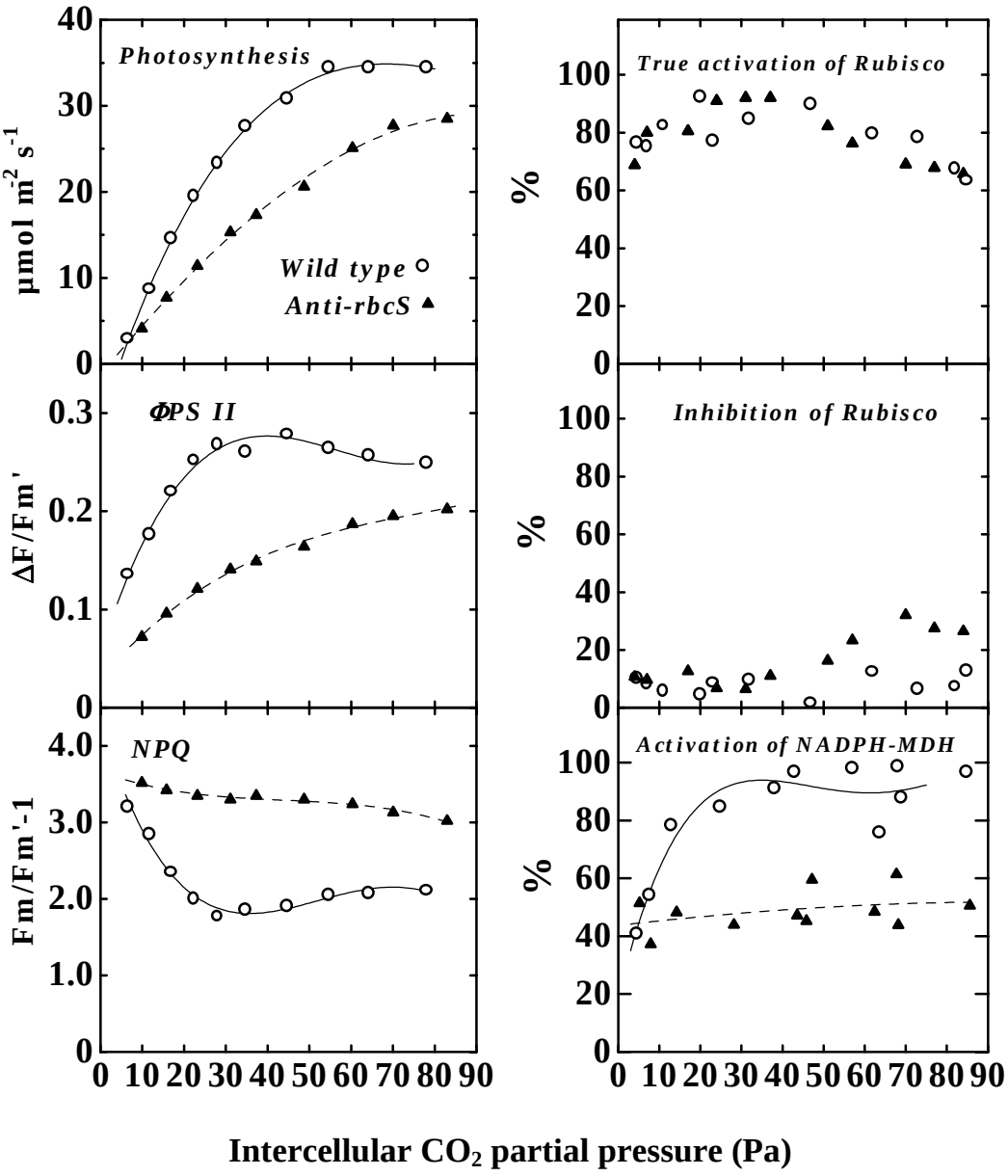

Fig. 1 Responses of the photosynthetic rate, ΦPS II, NPQ, Rubisco activation (initial activity/maximal activity), Rubisco inhibition (1 - total activity/maximal activity) and NADP-MDH activation to intercellular CO_2 pressure. Measurements were made at a leaf temperature of 25˚C, an irradiance of 1500 μmol m^{-2} s^{-1}.

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