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The regulation of photosynthetic rate in Cyanobacterium

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Introduction

In the higher plant, it is proposed that the CO_2 assimilation rate under high CO_2 concentration is limited by the rate of electron transport or RuBP regeneration. Cyanobacterial regulatory mechanism of photosynthesis is considered to be almost identical to that of C3 higher plant except CCM (CO_2 concentration mechanism). However, the regulation mechanism for CO_2 assimilation rate in photosynthetic Cyanobacterium has not been clear, yet.

There were a few reports, which showed the quantitative influence for RuBisCO reaction by the carboxysome, and Sato et al (1997) clarified the diffusion resistance of the carboxysome against Mg^{2+}, inorganic CO_2, PGA and RuBP. They prepared RuBisCO within carboxysome and in cytosol separately according to Price et al (1992), and evaluated the lag phase in a carboxylase reaction of each RuBisCO. From their results, nature of this carboxysome should be considered for photosynthetic rate regulation of Cyanobacterium.

Photosynthetic rates of a higher plant and Cyanobacterium change dependent on light intensity and CO_2 concentration, but under high light condition the rate depend on only CO_2 concentrations. Concerning a higher plant, such a photosynthetic rate change is regulated by an activation by CO_2 of RuBisCO and KmCO2 value under low CO_2 conditions and a regenerative rate of RuBP which is substrate of RuBisCO is thought to be a limiting step under low CO_2 conditions (von Cammerer and Edmondson. 1986). In other words, RuBP concentration decreases following increase of CO_2 concentrations,

and becomes finally lower than RuBisCO active site. As a result, concentration of RuBisCO-RuBP complex decrease and RuBisCO reaction rate become a steady state. On the other hand, PGA concentration rises following a rise of CO_2 concentrations. As PGA inhibits RuBisCO competitively to RuBP, inhibition of RuBisCO by PGA becomes noticeable under high CO2 concentration (Badger and Lorimer. 1981). Under high CO_2 conditions, RuBisCO reaction rate within a cell is limited by decrease of RuBP and increase of PGA concentration and the photosynthetic rate is limited as a result (von Cammerer and Edmondson. 1986). Thus the RuBisCO directly fixing CO_2 must be a controlle factor in photosynthesis, although there is no trial to increase the enzyme in a photosynthetic organism. In this research, RuBisCO activity was tried to increase within Cyanobacterium, and the primary aim of this study was to reveal the regulatory role of the enzyme in photosynthesis.

Materials and methods

Culture of cells

Synechococcus PCC7942 was cultured in BG-11 medium at 30˚C under cool white fluorescent light. Streptomycine (15 μg / ml) and chrolamphenicol (5 μg / ml) were added for culturing of HX and AIIX respectively.

HX and AIIX were produced by transformation with the shuttle vector pHX201and pNE205 respectively. The vector for the expression of cvrbc (pHX201 and PNE 205) were constructed in shuttle vector pUC303 by using 7942 psbAI promotor and 6803 psbAII promoter respectively.

Enzyme assay

Cells were grown in BG11 medium until OD730 of the medium reached around 1.0. Preparations of crude extracts were mainly according to the method of Price et. al. RuBisCO activity was measured spectrophotometrically.

Results

For high expression of a foreign protein within a cyanobacterium, 7942 psbAI promoters and 6803 psbAII promoters were effective. Then, expression vector (pHX201,

pNE205) of cvrbc was constructed in shuttle vector pUC303 by using the 2 promoters, respectively. When *C. vinosum* RuBisCO was expressed within cyanobacterium by the expression vectors, both vectors worked well to give HX, AIIX strain, respectively. Total activities of RuBisCO were 1.6 and 2.0 μmol RuBP/mg Chl/min in wild *S.* 7942 and the control strain with pUC303 plasmid, rescpectively. On the other hand, the values were 2.6, and 3.2 μmol RuBP/mg Chl/min for AIIX-15 and 17, while HX-10, 18, 12, and 11 showed the activities of 3.8, 4.7, 5.4, 6.7 μmol RuBP/mg Chl/min. Western blotting for L-subunit showed that *C. vinosum* RuBisCO was expressed in all strains. RuBisCO high expression mutant, HX, has 2~5 fold higher total RuBisCO activity than wild type has. The expression level of the exogenous RuBisCO gene is unstable, and its total RuBisCO activity decreased to the level of wild type RuBisCO activity during successive culture. On the other hand, the mutant which is transformed by *Chromatium vinosum* RuBisCO with *psb*AII promoter of *Synechocystis* PCC6803 is stable and the total RuBisCO activity was about 2 fold higher than that of the wild type. The relationship between the photosynthesis rate and the RuBisCO activities in wild, HX and AIIX mutant of *S.* 7942, showed that there seemed to be a different mechanism on the regulation of photosynthesis rate under the high CO_2 concentration from that of the higher plant, when the CCM may not be active. Even under the high CO_2 concentration, the photosynthesis of Cyanobacterium is limited by the total RuBisCO activity until the activity increased up to 2 fold of that of wild type. Then the photosystem activity decreased as the RuBisCO activity increased. As the results, the photosynthesis ability of the transformant of which total RuBisCO activity increased over 2 fold of the wild type activity decreased even under the high CO_2 concentration. A model simulation on computer was constructed to explain the phenomena with the GEPASI software.

Discussion

The *psb*AII promoter of *Synechocystis* PCC6803 was stable in *Synechococcus* PCC7942 to express *Chromatium vinosum* RuBisCO, and the instability of *psb*AI promoter of *Synechococcus* PCC7942 in the Cyanobacterium depended on the homologous recombination as expected.

In a cyanobacterium, *Synechococcus* PCC7942, the activity of photosystem I is much lower than that of RuBisCO. This means that the control coefficient of RuBisCO is high

even under high concentration of CO_2 in contrast with the case of the higher plant. However, the RuBisCO activity increased over 2 fold, the activity of the photosystem I seems to be downregulated. From model simulation, the photosynthetic oxygen evolution rate was explained with the RuBisCO activity, which worked, on inorganic carbon fixation except the activity for photorespiration.

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